Acta Medica Scandinavica

TABLE OF CONTENTS

VOLUME 202, 1977

SUPPLEMENTS TO VOLUME 202

604 Myocardial infarction in the elderly A clinical and epidemiological study with an one year follow up By V Konu

605 Cardiovascular complications in diabetics and subjects with reduced glucose tolerance By G Persson

606 Basle Hypertension Symposium Proceedings of a symposium held at Sandoz AG Basle December 13th and 14th 1976 Edited by H Dunér

The care of the hypertensive patient Treatment of hypertension in the community By G Berglund O Andersson and L Wilhelmsen Long term aspects on essential hypertension By I Liljefors Patient compliance By H Åberg *Haemodynamics in essential hypertension* Introduction By H Dunér Central haemodynamics in essential hypertension By P Lund Johansen Peripheral haemodynamics in essential hypertension By R Sivertsson Effects of beta adrenoceptor blocking agents on haemodynamic parameters By L Hansson Haemodynamic effects of pindolol in hypertensive patients By J H Atterhög H Dunér and B Pernow *Clinical pharmacology of beta adrenoceptor blocking agents* Pindolol A pharmacokinetic comparison with other beta adrenoceptor blocking patients By J H Atterhög H Dunér and B Pernow *Clinical pharmacology of beta adrenoceptor blocking agents* Pindolol A pharmacokinetic comparison with other beta adrenoceptor blocking agents By J Meier Investigations with beta adrenoceptor blocking drugs in healthy volunteers By W Aellig Once a day treatment of hypertension By G Frithz *Future development in the field of hypertension* Introduction By N Hökfelt New trends in pharmacology By M Henning Dopamine receptor stimulants in hypertension By B Clark The prevention of hypertension By G Tibblin and C G Eriksson

607 A new parallel plate dialyser I Development and testing of membrane support By S Dawids and C Boe II Description of design and in vitro performance By S Dawids and C Boe III Preliminary experience on clinical performance By S Dawids and S Vojnovic

608 Long term treatment with β_2-adrenostimulants in asthma Side-effects selectivity tolerance and routes of administration By S Larsson

609 Incidence and prevalence of endemic benign (epidemic) nephropathy in AC county Sweden in relation to population density and prevalence of small rodents By K Nyström

610 Cholesterol and bile acid metabolism in normo- and hyperlipoproteinaemia. By B Angelin

611 The gerontological and geriatric population study in Goteborg Sweden Seventy year-old people in Gothenburg A population study in an industrialized Swedish city II General presentation of social and medical conditions By A Svanborg Intake of energy and nutrients and meal habits in 70-year old males and females in Gothenburg Sweden A population study By B Steen B Isaksson and A Svanborg Body composition in 70-year-old males and females in Gothenburg Sweden A population study By B Steen Å Bruce B Isaksson T Lewin and A Svanborg

SUBJECT INDEX

(Supplements see p v)

Blood

Bone

Brain

Calcium

Catecholamines

Circulation

Coagulation

Complement

Cytology

Diabetes mellitus

Diagnosis

Diuretics

Electrolytes

Endocrinology

Enzymes

Exercise

Eye

Fatty acids

Fever

Gastrointestinal tract

Glucose

Heart

Hemodialysis

Heredity

Hormones

Hypertension

Immunology

Infection

Insulin

Intoxication

Lipoproteins

Liver

Lung

Lymphoma

Malformations

Metabolism

Platelets

Population studies

Pregnancy

Proteins

Respiration

Pulse

Rheumatic diseases

Sarcoidosis

Skin

Smoking

Syncope

Thrombosis

Thyroid

Transplantation

Treatment

Tuberculosis

Tumours

Uremia

Urinary tract

Urine

EDITORIAL

Technics and Ethics in Managing Acute Leukemia

he frequency of complete remissions in acute ıyeloblastic leukemia has increased from about 0% in 1965 up to in the best reports 70% in 1975 here is sometimes a tendency to attribute this to ew cytostatic combinations tested in randomized ulticenter studies of standardized treatment chedules and to forget that intensive supportive are is equally important

nfection

nitial infection is the second worst prognostic actor after high age (6) Early death usually be ause of septicemia prevents many patients from etting adequate cytostatic treatment improved upportive care reduced its frequency in our divi ion from 9/21 cases (43%) in 1971 to 2/18 (11%) in 1975 If the patient is afebrile when admitted pro-phylactic measures must be considered as soon as neutrophils fall below 0 15×10⁹/l Strict life island or laminar air flow isolation and/or gut sterilization have been claimed to be beneficial (6) as are very careful daily mouth skin and nail care Alternatively semi isolation including dustmats masks gowns and gloves is probably indicated We use prophylactic trimethoprim sulfa and lactulose orally but have not yet documented their usefulness Prophylactic antibiotics are not advisable

Once infection is established i e fever over 38 C has lasted for more than 4 hours immediate treatment is required Radiographic studies may involve a loss of time since the usual purulent reaction is often not developed by neutropenic patients One cannot wait for results of cultures since 50% of untreated neutropenic patients with severe infections die within 48 hours Unlike exogenous infections the endogenous infection is not primarily localized in an accessible organ of entry or a focus—such as the tonsils the respiratory or urinary tracts—from where the causative organism can be isolated Instead it enters the bloodstream from the gut Cultures can be obtained from the blood but the bacteremia is of short duration and negative cultures do not exclude septicemia

Antibiotics

Even if the causative microorganism cannot be cultivated treatment must not be blind Instead it must be based on experience and on knowledge of bacteria and fungi causing infection in neutropenic patients Few of these are sensitive to any of the ordinary spectrum penicillins alone In fact no single antibiotic can be considered adequate in this situation a combination is required In bacterial infections it is selected from a very limited group of agents including carbenicillin aminoglycosides cephalothin clindamycin and trimethoprimsulfa Among the aminoglycosides gentamycin is the most used Which combination should be selected is debatable it should be active against Gram negative Enterobacteriaceae notably E coli Proteus and Ps aeruginosa We start with carbenicillin and gentamycin which are both bactericidal and effective even in neutropenia They also cover staphylococci and H influenzae If fever has not subsided after 48 hours a third antibiotic from the group mentioned is added Clindamycin and high dose penicillin are useful in anaerobic infections where the Bacteroides group is common in leukemia patients

Between 20 and 50% of leukemic patients treated with modern antibiotics have systemic fungal infections at autopsy (2 10) So far laboratory studies cannot be relied on to establish the diagnosis of candidiasis Cultures if positive are relevant mainly when taken from blood where they may take up to 2 weeks to grow Although several hemagglutination and immunodiffusion techniques have been used to demonstrate antibodies against Candida the absence of a rise in the titer does not exclude systemic candidiasis Up to 36% false negatives have been reported in partly anergic leukemic

patients When visible superficial oral candidiasis was present before death systemic candidiasis was generally found at autopsy (2) Daily oral inspections are necessary and candidiasis should be suspected when there is oral candidiasis retrosternal pain or refractory low-grade fever for more than 4 days Oral mycostatin has been recommended to prevent fungal infections but is quite insufficient for the treatment of systemic candidiasis 5 fluoro-cytosine is frequently rapidly effective Perhaps resistance can be avoided with low doses of cell wall active amphothericin B (10) In the future assays of free so-called Mannan antigens could become a diagnostic alternative

Granulocytes

Where HLA matched siblings and continuous flow filtration or blood cell separators are available in the acute situation leucocyte concentrates containing about 10^{11} granulocytes can be obtained Transfusions of this kind are undoubtedly very valuable With this treatment 12 patients with Gram negative septicemia all survived (17) Lacking these facilities we use leukocyte concentrates from 6 blood units Only 10^{9} granulocytes are obtained in this way the recipient granulocyte count does not increase significantly and the value of these simplified granulocyte transfusions has not been documented

Bleeding

Hemorrhage was a feared and frequent complication earlier *Now it can usually be managed* Platelet concentrates from 6–8 units of ABO-Rh matched units of blood raise the recipient platelet count by about 11×10^{9}/l i e over the level of severe bleeding (9) We use them neither prophylactically nor if the only bleeding is petechial Patients who are not immunosuppressed seem to develop antibodies and only the first few transfusions are effective Cytostatic treated leukemia patients however seem sufficiently immunosuppressed to accept even HLA matched platelets up to at least 8 times without a demonstrable decrease in recovery (9)

Most lethal intracranial hemorrhages we have seen during the last few years occurred in patients with promyelocytic leukemia It is not yet known if the coagulopathy in these patients and in some of those with myeloblastic leukemia consists of disseminated intravascular coagulation or pri fibrinolysis In typical cases both fibrin mono- fibrinogen split products and a decrease in the centration of coagulation factors are found be acute risk of intracranial bleeding cannot be cluded if borderline or partially negative fi are made A lethal intracranial bleeding may o during the patient s first night in the hospital morphology of the leukemic cells must therefor established within hours All the vigilance intensive care must be used to detect ech and respiratory distress Often the risk of wa for the laboratory results may be greater than of starting unwarranted prophylactic tre Tranexamic acid alone has been found eff (13) but would not be expected to stop the p lated consumption of coagulation factors We t fore use 10000 units of heparin in a 24-hour in addition

Electrolytes

Calcium and potassium disturbances almost known a few years ago are receiving incre attention (8 11 14) It is not known if incr urinary potassium loss is caused by some an ics or by hyperaldosteronism secondary to h albuminemia We have seen occasional h calcemic tetany as well as muscular adyn intestinal paralysis polyuria and diabetes whether these are attributable to potassium d ency remains to be established

Cytostatics

Single cytostatics are unsatisfactory except promyelocytic leukemia In other forms of a leukemia a combination has to be selected fr limited group of antimetabolites alkaloids alk ing agents and anthracyclines No entirely ra philosophy of selecting the combinations exis yet but some principles do One is to comb S phase specific antimetabolites with cycle-ph non specific drugs (anthracyclines or alkyla another to combine drugs with different typ side-effects The differences in complete rem rate between different described combina con taining 2–4 drugs are smaller (3) than the di ences between different institutions using the sa combination (4) It is noteworthy that the frequen of remission can be twice as high in an instit

ere ntens ve supportive care is ava lable than in where it is not e en f both use dentical com at ons of cytostat cs (4)

The eth cs of test ng cytostat c comb nat ons n standard zed randomized programs be mes debatable unless nten ve s pportive care s first been made a a lable Such programs have n cla med to prevent some pat ents from gett ng e probably mo t effective treatment and to be so m ng that they delay introduct on of ch treatment for others (4) Cytostat cs are also ng our most toxic drugs and the scient fc vantage of standard zed dosage m st be e ghed a nst the advantage to the pat ent of nd v dual d do age After in t at ng treatment with a andard dose related to the body w e ght or surface complete rem ss on can somet mes be ach eved ter the first course of cytostat cs If not careful of pat ent and blood values frequent perm t nd v dual mod ficat ons of the dose n the ond course of treatment If platelets granulo- tes and le kem c blasts all d sappear after the rst dose f th s is follo ed by a fe er peak and if o s gns of normal cell regenerat on are seen the ose should probably be reduced If ne ther cell ype s affected it may be ncrea ed If only atelets and granulocytes are decreased after ? es but the leukem c cells are not a change of ytostat cs should be cons dered If the standard treatment schedule is carr ed through regard of the pat ent s react on both the length and qual ty of his rema n ng l fe may be endangered It is often ma nta ned that a str ctly sc ent f c test ng hedule is des rable to evaluate d fferent cytostat c omb nat ons However t has also been cla med hat a random zed schedule is not def n tely su to the use of h storical controls in stud es operly matched and strat f ed accord ng to prog ost c pretreatment variables such as infect on (5)

M nte ance f re ss o

Bo h the reported durat on f unma nta ned rem s s on and that of chemotherapy ma nta ned rem s s on ar es w dely (1 3) and st d es of poss ble reasons f r these variat ons are urgently requ red (3) The ariat ons make t d ff cult to e aluate the methods tested at present t prol ng em ss on namely monthly consol dat on ourses of cyto- stat cs early or late nten f cation of cytostat c reatment and mmunotherapy Immunotherapy can be spec fic us ng leukem c cells or non spec f c usually using BCG

Spec f c imm notherapy is based on t o con cepts one be ng that of ant gen c d fferences be t een leukem c and normal myeloblasts the other that of immunolog c tumor surveillance These concepts are st ll controvers al (1? 16)

Ho ever non spec f c immunotherapy may well be active All reports where non spec f c mmuno therapy was given together w th or without speci f c mmunotherapy seemed to sho v a benef c al though temporary effect (7 16)

Tl e q al t of l fe in l il at of death

If the d agnos s is made early the pat ent adm tted rap dly infect on followed and treated intens vely and cytostatics appl ed sk lfully and with luck it somet mes becomes possible to spare pat ents with leukem a in the n t al acute stage the sufferings of fever weakness nausea and pa n As soon as rem ss on has been ach eved even part ally the patient should be at home As soon as rem ss on is complete he should be g ven the opt on to resume travel stud es or w ork

During the f nal stages of the d sease hen the leukem a has relapsed and cytostat c treatment becomes ineff c ent an often neglected but very important phase of med cal care starts that of ensuring a reasonable qual ty of death Unthought ful fearful or unsk lful care can make fever weak ness nausea and pain unbearable

A dec s on must be made when to end attempts at cure and when to start term nal care D agnos t c measures and treatment a ming at a new re m ss on must then be replaced by pall ation wh ch has to be as nten e as the earl er treatment Anal ges cs and the r potent ators such as chlorpro- maz ne should be g en with sat sfactory fre quency and not only on demand Fever often re fractory and excruc at ng may react to ACTH particularly f caused by Cand da nfect on (5) Nausea should be treated intravenously and an i v channel be kept open Care must be taken that analges cs chlorpromaz ne and ant emet cs let the pat ent e ther sleep or be fully awake and that the exc tat on stage n between ith hyperacus a and eakness be avo ded So should other drug effects such as xerostom a The pat ent s wish regard ng food drug exam nat ons transfus ons v s ts etc should be la at th stage In add t on to stud es

of managing complications of acute leukemia in the early stage and those of inducing and maintaining remission many careful and considerate future studies are also required of the important art of ensuring a high quality of death

Peter Reizenstein Stockholm

ACKNOWLEDGEMENTS

Some studies mentioned were supported by the Swedish Cancer Society and Hoffman La Roche

REFERENCES

1 Bekesi J G Holland J F Fleminger R Yates J & Henderson E S Immunotherapeutic efficacy of neuraminidase treated allogeneic myeloblasts in patients with acute myelocytic leukemia Ann NY Acad Sci In press 1976
2 Chi C H Lagerlof B Lantz B & Reizenstein P Autopsy findings in leukemia Scand J Haematol 17 251 1976
3 Clarkson B D Dowling M D Gee T S Cunningham I B & Burchenal J H Treatment of acute leukemia in adults Cancer 36 775 1975
4 EORTC Comparison of the effect of cytosine arabinoside of cytosine arabinoside and thioguanine and of cytosine arabinoside and daunorubicine in acute myeloid leukemia (In French) Cooperative Leukemia and Hematosarcoma Group of the EORTC Bull Cancer (Paris) 61 411 1974
5 Freireich E J & Gehan E A Historical controls reincarnated An examination of techniques for clinical therapeutic research Guest Editorial Cancer Chemother Rep 58 623 1974
6 Freireich E J Gehan E A Speer J F H brun L Smith T Bodey G P McCredie K Rodriquez V Hart J S & Burgess M A usefulness of multiple pretreatment patient charac istics for prediction of response and survival in tients with adult acute leukemia Adv Biosci 14 1973
7 Hersh E M Gutterman J U & Mavligit G Immunotherapy of leukemia Med Clin North 60 1019 1976
8 Hocker P & Reizenstein P Calcium and potas disturbances in acute leukemia Blut 29 398 1974
9 — Effect on platelet counts and fever of pla transfusion in leukemia Blut 31 143 1975
10 Klastersky J Therapy of infections in a leukemia In Chemotherapy vol I Clinical asp of infections Proc 9th Int Congr Chemother London 1975 (ed J D Williams and A M Ged p 87 Plenum Press New York 1976
11 McKerr L Hypocalcemia in leukemia South M 68 828 1975
12 Moller E & Moller G The immune system an velopment of cancer (In Swedish) Läkartidn 73 3105 1976
13 Nilsson I M & Sjoerdsma A Antifibrino activity and metabolism of epsilon iminocaproi in man Lancet I 1322 1960
14 Parker A Plasma potassium levels in leuke Lancet I 392 1975
15 Reizenstein P Palliation of fever in leukaemia ACTH Opusc Med 21 125 1976
16 Reizenstein P Hoglund S Lindemalm Pauli C Udén A M Lehtinen T & Sjög A M Lymphocyte recognition of myeloblasts survival in myeloblastic leukemia given imm therapy Congr Med Oncol Soc Nice 1975 E Cancer To be published
17 Slyck E J Supportive care of the leukemic tient Henry Ford Hosp Med J 23 117 1975

Intermittent Melphalan and Prednisolone Therapy in Plasma Cell Myeloma

H Mellstedt M Bjorkholm and G Holm

From the Department of Medicine Karolinska Institutet at Serafimerlasarettet Stockholm Sweden

ABSTRACT Thirty two consecutive previously untreated patients with plasma cell myeloma were treated with 4 day courses of melphalan (0 25 mg/kg/day) and prednisolone (2 mg/kg/day) every sixth week The observation period ranged from 26 to 75 months and the total median survival time was 29 months 75% of the patients responded to therapy and their median survival time was 42 months Sex did not influence either the response rate or the survival time Most patients were treated in an out patient clinic and required a minimum of check ups

The survival of patients with multiple myeloma has greatly improved since the introduction of alkylating agents (30 31) The most useful drugs are melphalan and cyclophosphamide which are reported to be equally effective (25) These agents may cause clinical remissions accompanied by prolonged survival time and improved quality of life However there is much controversy regarding the efficiency of various drug regimens (2 3 4 5 9 11 13 14 16 17 20 24 25)

STUDY POPULATION AND METHODS

The study comprises 32 patients who fulfilled the diagnostic criteria of myelomatosis (see below) No patient had previously received any cytostatic or corticosteroid therapy The average age of the patients at the time of diagnosis was 66 years (range 48–84) and the sex distribution was about 1 1 (Fig 1) All patients were seen at the Department of Medicine Serafimerlasarettet Stockholm from March 1 1970 to March 31 1974 The survival analysis was performed on May 31 1976 The follow up ranged from 26 to 75 months

The diagnosis of myelomatosis was established if two or three of the following criteria were met 1) More than 10% plasma cells in aspirated bone marrow specimens 2) A myeloma globulin peak on serum and/or urine electrophoresis in association with decreased concentration of normal serum immunoglobulins (Agarose electrophoresis was performed and urine was concentrated 50–200 times immunoglobulins were determined either by the radial immunodiffusion technique according to Mancini (23) or by the rocket technique according to Laurell (19)) This criterion is compulsory for the diagnosis 3) Osteolytic and/or osteoporotic bone lesions demonstrated by X ray examination of the skull vertebral column and pelvic bones

When the diagnosis had been confirmed all patients received melphalan 0 25 mg/kg b wt /day and prednisolone 2 mg/kg b wt /day orally for four days (4) The steroids were discontinued promptly The course was repeated every sixth week indefinitely If severe hematologic toxic side-effects were noted (WBC<1 000/μl and platelet count <50 000/μl) the interval was prolonged or exceptionally the dosage was reduced Localized radiotherapy for symptomatic bone lesions was given when indicated

The patients were followed up at regular intervals by repeated measurements of Hb concentration WBC and platelet counts ESR serum creatinine and serum calcium Serum immunoglobulin concentration urinary protein excretion (g/day) quantitated by the Biuret technique (after electrophoresis had demonstrated that most of the material consisted of myeloma protein) and the percentage of plasma cells in bone marrow were determined 2–3 times a year

Response to therapy was defined as follows 1) Decrease in serum myeloma globulin concentration to less than 50% of the pretreatment value and to less than 4 0 g/100 ml 2) Decrease in urinary protein excretion (see above) to less than 50% of the pretreatment value and to less than 0 5 g/day In addition clinical improvement was required

Survival time was established by the life table method according to Cutler and Ederer (10)

RESULTS

The distribution of the patients by immunoglobulin class of the M component is shown in Table I and

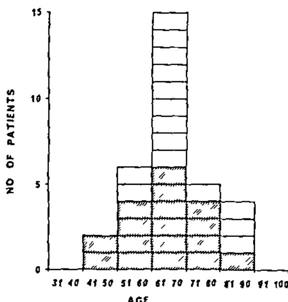


Fig 1 Age and sex distribution ▨=male □=female

some initial laboratory findings are presented in Table II

No correlation was seen between routine *hematological tests and immunoglobulin classes* Six of the nine patients with light chain myelomas had an initial serum creatinine level of more than 2 1 mg/100 ml (mean value for these six patients 4 0 /100 ml) while the serum creatinine level never exceeded 1 9 mg/100 ml in patients with IgG and IgA myelomas

As soon as the diagnosis had been confirmed therapy was instituted (see above) After approximately four courses the WBC had stabilized to values around 3 000–3 500/μl The rate of objective response was 75% (23/32) 80% of the IgG myelomas and 89% of the light chain myelomas responded to therapy but only 50% of the IgA

Table I *Distribution of myeloma patients by immunoglobulin class and response to therapy*

	IgG myeloma	IgA myeloma	Light chain myeloma		Total
			κ type	λ type	
No of pats	*15*	*8*	7	2	32
%	47	25	22	6	
No of pats responding to therapy	12	4	6	2	24
%	50	17	25	8	

Table II *Initial laboratory findings*

	N	%	Mean±S[illegible]
Anemia (Hb <11 g/100 ml)	19	59	10 7±2 6
Leukopenia (WBC <3 0×10³/ml)	7	22	5 7±[illegible]6
Thrombocytopenia (platelet count <150×10³/ml)	7	22	[illegible]07±73
Serum creatinine >1 2 mg/100 ml	15	47	1 7±1 3
Hypercalcemia (>5 3 mEq/100 ml)	9	28	5 [illegible]±0 8
ESR >25 mm/h	30	94	87±47

myelomas (Table I) No difference in response ra[te] was observed between males and females

The total median survival time was 29 mon[ths] and the 5 year survival was 14% (Fig 2) Amo[ng] responding patients only the survival curve show[s] that approximately 50% are alive at 42 months an[d] the 5 year survival is 19% Among the 8 no[n] responders the survival time ranged from 7 to [illegible] months

No difference in the survival time was seen be[tween] *men and women Since the material is lim*ited no attempt has been made to compare th[e] survival times for patients in relation to paraprote[in] types However it should be mentioned that tw[o] patients with light chain myelomas of κ type are sti[ll] alive after 74 and 33 months They had an initi[al] serum creatinine level of 2 1 and 4 3 mg/100 ml respectively One patient with a λ type light cha[in] myeloma with an initial serum creatinine value o[f] 1 1 mg/100 ml died after 60 months

The toxicity of the drug program was low Mo[st] patients developed a mild leukopenia and o[r] thrombocytopenia within two weeks from a cours[e] but WBC and platelet counts had returned by the time the next course of treatment was due to start *In three patients the melphalan dosage had to be* reduced because of bone marrow depression All three had an elevated serum creatinine level The bone marrow depression was never severe One patient developed a moderate diabetes which was well controlled by oral medication and did not call for any reduction of the steroids In one patient a duodenal ulcer with bleeding was noted 4 weeks after the end of a course In three patients the steroid dosage was reduced slightly on account of mental discomfort

During the observation period 23 out of 32 patients died two from an acute myocardial infarc[tion]

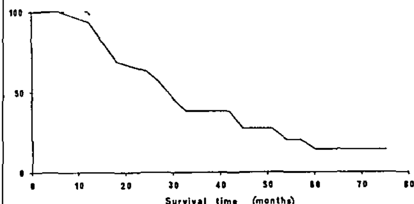


Fig 2 Survival curves
—=all patients
responding patients

on with no signs of active myelomatosis The ther 21 deaths were related to myeloma mostly as idespread myeloma infiltration often in combina on with infections (pneumonia or sepsis) and/or leeding due to thrombocytopenia The main cause f four deaths (2 men and 2 women) was renal nsufficiency Eight deaths (7 women) were due to nfections On the other hand five of the six pa ents who died from hemorrhagic diathesis were nen No patient died in the classical clinical picture f plasma cell leukemia However six autopsies howed a diffuse scattered or nodular infiltration of ymphocytes and plasma cells throughout the body ncluding most parenchymatous organs (lungs iver kidneys pancreas lymph nodes and mentum) (5 6)

DISCUSSION

Thirty two patients with multiple myeloma have been treated with intermittent high dosages of melphalan and prednisolone The observation pe riod ranged from 26 to 75 months and 75% of the patients responded to therapy as defined by our a (see above) The total median survival time 29 months and the 5 year survival 14% 47% of responding patients were alive 42 months after iagnosis and 19% after 5 years

The results of this type of therapeutic regimen are good as those reported by other authors Com son with other studies regarding response rate however complicated by the use of different for evaluating the response Moreover it should be borne in mind that patients who do not fulfil given criteria still benefit from therapy i e they show no signs of progression of the disease and have a prolonged survival (32) Therefore today the most reliable parameter for evaluating the therapeutic effect is survival time

Hoogstraten et al (17) using melphalan in an initial loading dose followed by a continuous low dosage reported a median survival time of 23 months from institution of therapy Later Hoogstra ten et al (16) presented results from a study with intermittent melphalan therapy with a median survival time of 26 months McArthur et al (24) using only continuous melphalan therapy showed a median survival time of 26 months after institution of therapy (31 months from diagnosis) In the MRC study from 1971 (25) a loading dose of melphalan and continuous maintenance therapy gave a median survival time of only 18 months Alexanian et al (4) using the same treatment schedule and similar criteria as we do reported a response rate of 73% and a median survival time of 24 months Combina tions of melphalan–prednisolone–procarbazine and melphalan–prednisolone–procarbazine–vincristine have also been used and gave a median survival time of 24 months and 26 months respectively (2) George et al (13) who used the same treatment regimen and the same criteria for response as we reported a response rate of 74% None of their patients on the intermittent high-dosage melpha lan-prednisolone therapy died within the first six months just as in our study no patient died within the first seven months Costa et al (9) reported a significantly better response rate with a melphalan and prednisolone treatment schedule than with melphalan alone for good risk patients (55 versus 23%) as well as a longer survival time (53 versus 30 months) but poor risk patients showed no differ ence in response and their survival time was

for the combination program (9 months versus 21 months) The longest median survival time (40 months) is reported by Farhangi and Osserman (11)

In our experience intermittent high dose melphalan and prednisolone is not only an efficient but also a safe treatment with a low toxicity besides being easy to carry out The patients require a minimum of ambulant check ups once the disease has stabilized the patient can take every second course at home and needs a check up only once in 12 weeks It is also our impression that the need for supportive radiotherapy is minimized during this type of therapy Only one patient received X ray therapy as also was reported by George et al (13)

Our material is too small for a detailed analysis of the response in relation to myeloma subtypes However it is noteworthy that 89% of the light chain myelomas responded to therapy and that their survival was comparable with that of the other myeloma patients A high response rate (83%) in the light chain disease has also been reported by others (11) Different responsiveness among males and females (31 32) was not seen in our material

None of the patients had clinical signs of os ...osis which could be related to the therapy ... course of corticosteroids is probably too short ...se this side effect Furthermore bone resorp... in myeloma appears to result from the activation of osteoclasts by osteoclast activating factor (OAF) produced by the bone marrow myeloma cells (27) and prednisolone blocks the activation of OAF (28)

There also seems to be a theoretical justification for the use of corticosteroids Myelomatosis is a B lymphocyte malignancy We and others have shown that peripheral blood of myeloma patients contains lymphocytes with surface immunoglobulin structures characterized by the idiotypic structure of their myeloma protein and produced by the lymphocytes themselves (1 21 26) The monoclonal lymphocytes vary entirely with dissemination of the disease They may be progenitors to the malignant plasma cells and feeders to the malignant plasma cell pool Thus myelomatosis might be regarded as a lympho-plasma-cellular malignancy with circulating leukemic lymphoid cells (15) Corticosteroids have been shown to exert toxic effects on leukemic lymphocytes and perhaps also on normal human lymphocytes (8 12 18 22 29) Thus by using prednisolone in combination with melphalan in the treatment of myelomatosis a better cytostatic effect on the malignant cell pop... tion could be expected Moreover corticost... may relieve the suppression of the normal ant... synthesis in myelomatosis (7)

The prognosis in myelomatosis has however improved in recent years even with more aggres... drug combinations (2 5 14 20) New therap... approaches should be tried Hopefully new ...tostatics may become available and perhaps ...munologic treatment might be of value

ACKNOWLEDGEMENT

This study was supported by grants from Stock... County Council

REFERENCES

1 Abdou N I & Abdou N L The monoclonal ... of lymphocytes in multiple myeloma Ann Intern ... 83 42 1975
2 Alexanian R Balcerzak S Haut A Hewlett ... Gehan E Remission maintenance therapy for ...ple myeloma Arch Intern Med 135 147 1975
3 Alexanian R Bonnet J Gehan E Haut A ...lett J Lane M Monto R & Wilson H Com...tion chemotherapy for multiple myeloma C... 30 382 1972
4 Alexanian R Haut A Khan A U Lane ... McKelvey E M Migliore P J Stuckey W ... Wilson H Treatment for multiple myeloma C...nation chemotherapy with different melphalan regimens JAMA 208 1680 1969
5 Bergsagel D E & Pruzanski W Treatme... plasma cell myeloma with cytotoxic agents An...tern Med 135 172 1975
6 Bjorkholm M Holm G Mellstedt H & Sjo... A Extensive nodular infiltration of extra-os... tissues in human myelomatosis Acta Med ... 200 139 1976
7 Broder S Humphrey R Durm M Blackman ... Meade B Goldman C Strober W & Wald... T Impaired synthesis of polyclonal (non par...tein) immunoglobulins by circulating lympho... from patients with multiple myeloma N Engl J 293 887 1975
8 Claman H N Corticosteroids and lymphoid cel... Engl J Med 287 388 1972
9 Costa G Engle R L Jr Schilling A Cabon... Kochwa S Nachman R L & Glidewell ... Melphalan and prednisone An effective combin... for the treatment of multiple myeloma Am J ... 54 589 1973
10 Cutler S J & Ederer F Maximum utilization ... life table method in analyzing survival J Chro... 8 699 1958
11 Farhangi M & Osserman E F The treatme... multiple myeloma Semin Hematol 10 149 1973
12 Fauci A S Mechanisms of corticosteroid actio...

lymphocyte subpopulations Clin Exp Immunol 24 54 1976
13 George R P Poth J L Gordon D & Schrier S L Multiple myeloma—intermittent combination chemotherapy compared to continuous therapy Cancer 29 1665 1972
14 Hammack W J Huguley C M Jr & Chan Y K Treatment of myeloma Comparison of melphalan chlorambucil and azathioprine Arch Intern Med 135 157 1975
15 Holm G Mellstedt H Pettersson D & Biberfeld P Idiotypic immunoglobulin structures on blood lymphocytes in human plasma cell myeloma Immunol Rev 34 139 1977
16 Hoogstraten B Costa G Cuttner J Forcier J Leone L A Harley J B & Glidewell O J Intermittent melphalan therapy in multiple myeloma JAMA 209 251 1969
17 Hoogstraten B Sheehe P R Cuttner J Cooper T Kyle R A Oberfield R A Townsend S R Harley J B Hayes D M Costa G & Holland J F Melphalan in multiple myeloma Blood 30 74 1967
18 Killman S A Kinetics of leukemia cells in relation to treatment Nordic Cancer Union Åbo 1976
19 Laurell C B Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies Analyt Biochem 15 45 1966
20 Lee B J Sahakian G Clarkson B D & Krakoff I H Combination chemotherapy of multiple myeloma with alkeran cytoxan vincristine prednisone and BCNU Cancer 33 533 1974
21 Lindstrom F D Hardy W R Eberle B J & Williams R C Jr Multiple myeloma and benign monoclonal gammopathy differentiation by immunofluorescence of lymphocytes Ann Intern Med 78 837 1973
22 Lippman M E Halterman R H Leventhal B G Perry S & Thompson E B Glucocorticoid binding proteins in human acute lymphoblastic leukemic blast cells J Clin Invest 52 1715 1973
23 Mancini G Vaerman J P Carbonara A D & Heremans J F A single radial-diffusion method for the immunological quantitation of protein In XI Colloquium on Protides of Biological Fluids (ed H Peters) pp 370–373 Elsevier Amsterdam 1964
24 McArthur J R Athens J W Wintrobe M A & Cartwright G E Melphalan and myeloma Experience with a low-dose continuous regimen Ann Intern Med 72 665 1970
25 Medical Research Council Myelomatosis Comparison of melphalan and cyclophosphamide therapy Br Med J 1 640 1971
26 Mellstedt H Hammarstrom S & Holm G Monoclonal lymphocyte population in plasma cell myeloma Clin Exp Immunol 17 371 1974
27 Mundy G R Raisz L G Cooper R A Schlechter G P & Salmon S E Evidence for the secretion of an osteoclast stimulating factor in myeloma N Engl J Med 291 1041 1974
28 Raisz L G Luben R A Mundy G R Dietrich J W Horton J E & Trummel G L Effect of osteoclast activating factor from human leukocytes on bone metabolism J Clin Invest 56 408 1975
29 Waddell A W Bird C C & Currie A P Inhibition of uridine uptake by methyl prednisolone in human lymphoblastoid cells Biochem Soc Transact 4 262 1976
30 Waldenstrom J Melphalan therapy in myelomatosis Br Med J 1 859 1964
31 — Diagnosis and treatment of multiple myeloma Grune & Stratton New York 1970
32 — Personal communication 1976

Acta Med Scand 202 11-15 1977

Alcohol Consumption and Hematology

Mårten Myrhed Lena Berglund and Lars Erik Böttiger

From the Department of Clinical Alcohol and Drug Research and the Department of Medicine Karolinska Hospital Karolinska Institute Stockholm Sweden

ABSTRACT A number of hematological variables have been investigated and followed during a hospital stay in a group of 34 non-cirrhotic male alcoholics after acute drinking bouts The most prominent findings were a rise in reticulocytes, a fall in serum iron and a rise in WBC, especially with respect to the lymphocytes HB and hematocrit values both fell during hospitalization while ESR and serum haptoglobin rose No change was observed in the platelet count It is concluded that alcohol has marked effects on the hematological system even in subjects without serious liver damage The results underline the importance of an adequate knowledge of the patient s alcohol habits in the investigation of obscure hematologic abnormalities

Alcohol ingestion (alcohol is used here as a synonym for ethanol) has been implicated in the development of a number of hematological abnormalities (3) including vacuoles in erythroblasts (8 9) megaloblastic anemia (8 15) transient hemolysis with hyperlipidemia (16 17) a reversible type of sideroblastic erythropoiesis (6 7) and thrombocytopenia (4 9) Changes in the myelopoiesis have been reported as well (1 2 11) Most of these studies have been performed either in vitro in laboratory volunteers in skid row alcoholics or in subjects with alcohol induced liver cirrhosis

The purpose of the present investigation is to report on changes in a number of hematological variables during a hospital stay in alcohol-dependent subjects without serious liver damage

STUDY BASE

The present subject group was collected at the Department of Clinical Alcohol and Drug Research Karolinska Hospital A total of 34 consecutively admitted male patients aged 20–61 years (mean 41 S D 10) were studied (Table I) None had or developed delirium tremens while in the hospital and the majority were gainfully employed and apparently socially well adapted According to the policy of the Department all subjects were admitted voluntarily Nobody was suffering from hepatic complications such as cirrhosis of the liver The liver function tests are shown in Table II No patient had attracted particular hematologic interest earlier and all denied ingestion of unusual beverages such as cooking fluids or rubbing alcohol None had taken any medications known to induce hematological complications The alcohol intake has been assessed in grams of absolute alcohol ingested per day as described previously (12) The mean daily intake during the weeks before admission amounted to 244±102 g (250 g=75 cm^3 of hard liquor)

Blood samples were drawn on the day of admission on the seventh day and if the patient was still hospitalized after three weeks All analyses were performed at the Department of Clinical Chemistry Karolinska Hospital with their routine methods In the presentation of the separate variable the number of subjects varies This is due to the fact that only part of the material was analysed with respect to some variables in a few cases it is due to lost samples The mean intrapair differences were examined using Student s paired *t* test (13)

RESULTS

As it was considered of interest to see whether or not a high alcohol intake before admission gave pronounced hematologic changes the subjects were assessed in relation to daily consumption This approach showed only weak correlations and consequently the results have been pooled for the whole group

Erythropoiesis

Hb values on admission and after one week s hospital stay were 148 and 141 g/l respectively (Table III) Hematocrit showed a mean of 43 6% on the

Table I *Age distribution*

Age (y)	*n*
20–30	4
31–40	11
41–50	13
51–60	5
>60	1
Total	34

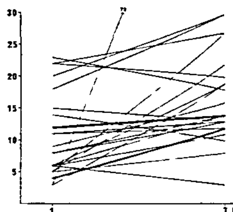


Fig 1 Changes in reticulocyte levels during hospital

first day and 41 9% a week later The subjects who were in-patients for 3 weeks did not show a return to the admission values No significant differences were found in erythrocyte counts nor in MCHC and MCV

The greatest disparities were found with respect to reticulocytes and serum iron (Table III) A normal mean value of about 1% for reticulocytes was observed on admission but this rose to 1 85% during the first 7 days (Fig 1) In the rather small group of 9 subjects followed for 3 weeks the mean decreased to 1 19% There was a highly significant fall in serum iron from 27 5 to 17 7 μmol/l during the first week (Table III) The same tendency was found among those followed for 3 weeks It may be ted that 4 out of 22 subjects had levels ≥50 l/l on admission (Fig 2)

Myelopoiesis

From the present study it is obvious that ethanol ingestion affects the white blood picture Thus the WBC was 6 26 on admission and 7 20×10⁹/l after 7 days in hospital (Table IV) *About the same difference between means* was observed for those who were in patients for 3 weeks Although there was a rise in the number of neutrophils in both groups it was not significant This implies that the gre differences were found for lymphocytes Out c subjects 19 had a lower value on admission tha the seventh day the means being 1 85 and 2 10⁹/l respectively A similar trend was obse in the group kept off ethanol for at least 3 week

Platelet count and ESR

No significant differences were observed in mean values of platelet counts (Table IV) Five of 26 patients however had thrombocytopenia 110 106 117 and 115×10⁹/l) on admission In five the values became normal within the first w

From Table IV it can be observed that ES lower immediately after the cessation of drin than during the following weeks the present me being 9 on admission and 14 mm after one wee hospital Similar tendencies were seen among th *who remained in hospital for the longer period* levels in this group being 10 and 16 mm resp tively

Haptoglobin values were strongly affected

Table II *Liver function tests in alcohol-dependent subjects*

Mean values S D given within parentheses

	On admission	After 1 week	*n*	Reference values
S ASAT (μkat/l)	1 53 (1 11)	0 82 (0 50)	28	0 20–0 70
S-ALAT (μkat/l)	0 96 (0 57)	1 01 (0 61)	27	0 10–0 70
S-LD (μkat/l)	8 70 (2 33)	6 53 (1 25)	28	3 5–7 0
S ALP (μkat/l)	3 84 (1 78)	3 03 (1 16)	28	0 8–4 0
S-GT (μkat/l)	5 98 (8 90)	4 18 (5 56)	25	0 1–1 0
S-bilirubin (μmol/l)	9 0 (5 9)	5 9 (3 2)	28	0–21

*$p<0.01$ * $p<0.001$

able III *Erythropoiesis in alcohol-dependent subjects*
lean values S.D given within parentheses

	On admission		After 1 week		n	On admission		After 3 weeks		n	Reference values
IB (g/l)	148	(16)	141	(13) *	32	145	(19)	138	(11)	12	140–170
lematocrit (%)	43 6	(3 8)	41 9	(3 7)* *	32	43.2	(4 7)	41 1	(3.2)	12	42 50
×10 /l	4.50	(0 49)	4.31	(0 60)*	30	4.57	(0 49)	4 40	(0.37)	11	4.5–5.5
ICHC (g/l)	339	(19)	336	(12)	31	337	(21)	335	(10)	11	320–360
ICV (fl)	98	(10)	98	(11)	29	95	(9)	95	(9)	11	76–96
leticulocytes (%)	1 03	(0 64)	1 85	(0 13) *	25	0 98	(0 7)	1 19	(0 49)	9	0 2–2.0
erum iron (µmol/l)	27.5	(15 6)	17 7	(6.3) *	22	24 1	(17 6)	13.5	(6.0)*	7	14–32

$p<0.05$ $p<0.01$ * * $p<0.001$

thanol (Table IV) The means in the one week group were 1 24 on admission and 1 70 g/l after 7 days alcohol abstinence

olate and vitamin B_{12} levels

There were no changes in serum folate or serum vitamin B_{12} levels during the periods followed (Table IV) Observed means for serum folate were normal throughout while those of vitamin B_{12} were somewhat higher than the values usually reported as normal by the hospital laboratory

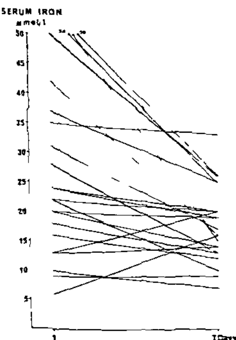


Fig 2 Changes in serum iron levels during hospital stay

DISCUSSION

The deleterious influence of ethanol on the liver the pancreas and the heart has been known for a long time while the first reports concerning the effects on the hematologic system appeared around 1960 (17) As most of the studies of hematologic damage have been performed either in vitro in laboratory volunteers in skid row alcoholics or in subjects with alcohol-induced liver cirrhosis it was considered of major interest to focus on a group of alcohol-dependent subjects without serious liver damage *Judging by the results of the present in*vestigation it is obvious that alcohol effects quite a number of hematological variables even in such a group

Anemia is for many reasons frequently found in alcoholic patients (3) Of major causes gastrointestinal bleeding extracorpuscular hemolysis chiefly localized to the spleen, but also a direct toxic effect of alcohol on the erythropoiesis in the bone marrow may be mentioned (10) In the present study anemia was not a common finding in the group either on admission or during the hospital stay However the effects of alcohol on Hb and hematocrit values could be clearly observed as both the means decreased significantly after the cessation of drinking These changes are probably mainly ascribable to the diuretic actions of ethanol (14) as is the effect on the ESR.

Megaloblastic anemia in alcoholics has been described by e g Hines (6) and Eichner and Hillman (5) In the presen stud, no definite conclusions concerning this hematologic abnormality can be drawn as the bone marrow of the subjects was not studied but the high MCV values in the peripheral blood despite normal folate and B_{12} levels are

Table IV *Myelopoiesis and some other hematologic variables in alcohol-dependent subjects*
Mean values S D given within parentheses

	On admission		After 1 week		n	On admission		After 3 weeks		n	Reference values
WBC×10⁹/l	6 26	(2 43)	7 20	(2 08)*	32	6 95	(2 48)	8 26	(3 23)	12	4–9
Segmented leucocytes×10⁹/l	3.34	(1 84)	3 62	(1 27)	22	4 01	(1 97)	4.55	(2 44)	11	1 6–6 3
Lymphocytes×10⁹/l	1 85	(0 72)	2 48	(0 89)***	22	1 95	(0.50)	2 45	(0 95)*	11	0 8–4 1
Platelets×10⁹/l	229	(82)	234	(86)	27	232	(74)	227	(88)	9	150–400
ESR (mm/h)	9	(5)	14	(9)**	28	10	(5)	16	(19)*	12	2–10
Haptoglobin (g/l)	1.24	(0 71)	1 70	(0 71)* *	25	0 91	(0 62)	1 41	(0 64)	10	0 4–1 8
Vitamin B_{12} (pmol/l)	759	(290)	724	(242)	26	878	(376)	766	(328)	13	110–660
Folate (nmol/l)	14	(8)	17	(9)	25	15	(10)	15	(7)	12	7–40

* $p<0.05$ ** $p<0.01$ *** $p<0.001$

ble However no reduction in the size of RBC appeared during the time in hospital

The most prominent findings concerning the erythropoiesis in the present study are in respect of reticulocytes and serum iron which both changed significantly This is in accordance with earlier studies reporting increased serum iron levels during the period of alcohol intake but a fall after its termination (5 7) It is obvious that alcohol disturbs the erythropoiesis even in these rather healthy alcohol-dependent subjects but that it is soon restored after the cessation of alcohol intake and is followed by a reticulocytosis and a fall in serum iron

The present study clearly shows that ethanol affects the white blood picture as well Thus a reduction was found with respect to total WBC segmented leucocytes and lymphocytes The most marked decrease appeared in lymphocytes The results are of interest as rather few reports dealing with leucopoiesis have been published Alcoholics have been reported to be more susceptible to pneumonia than the general population (11) and as a reduced leucocyte response after an injection of endotoxin was demonstrated in that study it was concluded that alcoholics have a diminished bone marrow reserve of leucocytes Alcohol has also been shown to increase susceptibility to experimental infections in animals and was found to produce a profound depression in the rate of leucocyte mobilization into traumatized skin lesion in normal people (2) However the phagocytic ability of the leucocytes was unaffected by alcohol in the latter study

There was no relationship between alcohol intake and a depressed mean platelet level on admission in the present study However five subjects display[ed] pathologically low values on admission They [all] recovered during the first week in hosp[ital] Thrombocytopenia has frequently been observed [in] study populations of alcoholics and has usual[ly] been considered to be a complication of cirrhosis [or] attributed to inadequate dietary intake of folic ac[id] Figures varying from 14% (9) to 81% (4) have be[en] reported Lindenbaum and Hargrove (9) describ[ed] 10 episodes of thrombocytopenia in five chron[ic] alcoholics with delirium tremens In all cases t[he] platelet count rose rapidly returning to norm[al] within 3–7 days and was followed by a subseque[nt] *thrombocytosis* When ethanol was administered [to] human volunteers the platelet count was marked[ly] depressed during the 3rd–5th week of alcohol inge[s]tion in four of nine subjects (10) At present it m[ay] be assumed that the effects of alcohol are twofol[d] firstly by a suppression of the production in bo[ne] marrow and secondly a shortening of the surviva[l] time (3)

There were no indications of reduced levels o[f] vitamin B_{12} and folate in the present subject gro[up] Concomitant folate deficiency macrocytosis an[d] other hematological abnormalities in alcoholic[s] have been described by some authors (6) It ha[s] been reported that some alcoholic subjects with [a] decreased serum folate activity have abnormal[ly] low values of serum vitamin B_{12} activity but h[igh] values of serum vitamin B_{12} could be observed at a[ll] levels of serum folate as well

To sum up the present report deals w[ith] *hematological effects in a group of non-cirrhotic* men with a high alcohol intake The results indica[te] that significant changes are found with respect t[o]

eticulocytes serum iron Hb hematocrit WBC ncluding segmented leucocytes lymphocytes ESR and haptoglobin The results underline the im portance of an adequate knowledge of the patient s habits in the investigation of obscure hematologic abnormalities

REFERENCES

1 Beard J D & Knott D H Hemopoietic response to experimental chronic alcoholism Am J Med Sci 252 518 1966
2 Brayton R G Stokes P E Schwartz M S & Louria D B Effect of alcohol and various diseases on leukocyte mobilization phagocytosis and intracel lular bacterial killing N Engl J Med 282 123 1970
3 Böttiger L. E Alcohol and the blood Scand J Haematol 10 321 1973
4 Cowan D H & Hines J D Thrombocytopenia of severe alcoholism Ann Intern Med 74 37 1971
5 Eichner E R & Hillman R S The evolution of anemia in alcoholic patients Am J Med 50 218 1971
6 Hines J D Reversible megaloblastic and sideroblas tic marrow abnormalities in alcoholic patients Br J Haematol 16 87 1969
7 Hourihane D O B & Weir D G Suppression of erythropoiesis by alcohol Br Med J 1 86 1970
8 Jarrold T Will J J Davies A R Duffey P H & Bramschreiber J L Bone marrow-erythroid mor phology in alcoholic patients Am J Clin Nutr 20 716 1967
9 Lindenbaum J & Hargrove R L Thrombo-cytopenia in alcoholics Ann Intern Med 86 526 1968
10 Lindenbaum J & Lieber D S Hematologic effects of alcohol in man in the absence of nutritional defi ciency N Engl J Med 281 333 1969
11 McFarland W & Libre E P Abnormal leucocyte response in alcoholism Ann Intern Med 59 865 1963
12 Myrhed M Alcohol consumption in relation to factors associated with ischemic heart disease Acta Med Scand (Suppl) 567 1974
13 Siegel S Nonparametric statistics McGrawhill New York Toronto and London 1956
14 Wallgren H & Barry H III Actions of alcohol part I-II Elsevier Amsterdam London and New York 1970
15 Waters A H Morley A A & Rankin J G Effect of alcohol on hemopoiesis Br Med J 2 1565 1966
16 Westerman M P Balcerzak S P & Heinle E W Red cell lipids in Zieve s syndrome Their relation to hemolysis and to red cell osmotic fragility J Lab Clin Med 72 663 1968
17 Zieve L Jaundice hyperlipemia and hemolytic anemia A heretofore unrecognized syndrome as sociated with alcoholic fatty liver and cirrhosis Ann Intern Med 48 471 1958

Acta Med Scand 202 17–21 1977

Cellular Immunocompetence in Asymptomatic Paraproteinaemia

J Weits G C de Gast T H The M T Esselink and E Mandema

From the Department of Internal Medicine University of Groningen Groningen the Netherlands

ABSTRACT Several sensitive parameters of cellular immunity were studied in 20 patients with asymptomatic paraproteinaemia as judged after at least 3 years of follow up In vitro lymphocyte transformation by the primary immunogen haemocyanin of Helix pomatia (HPH) following immunization by PHA PWM and previously encountered antigens was measured as was skin sensitization by the primary immunogen dinitrochlorobenzene (DNCB) All 12 patients with low serum paraprotein levels—benign paraproteinaemia according to common criteria—had normal parameters of cellular immunity However, 5 of 6 patients with high IgG and IgA serum paraprotein levels had a decreased response to HPH, 3 of these 5 also had a decreased PHA response Two patients with high IgM serum paraprotein levels had normal lymphocyte reactivity Lymphocyte reactivity by PWM and previously encountered antigens was normal in all patients Autologous serum culture medium did not influence the results DNCB skin sensitization was significantly lower in the group with high serum paraprotein levels than in the group with low levels It is concluded, that in asymptomatic paraproteinaemia patients with high serum paraprotein levels differ from those with low paraprotein levels in their defective cellular response to primary immunogens The significance of these findings is discussed

In multiple myeloma and Waldenstrom s macroglobulinaemia not only a depressed humoral immune response was found but also—unexpectedly—signs of a decreased cellular immunity (2 5 6 10 13 16 17) Defects in cellular immunity in paraproteinaemia could have allowed the development of an abnormal plasma cell clone Studies in asymptomatic paraproteinaemia may contribute to the clarification of this point

Such studies with several parameters of cellular immunity are not known to us Therefore we selected 20 patients with asymptomatic paraproteinaemia of whom 12 had benign paraproteinaemia as judged by common criteria (4 12 15 18) and 8 had high serum paraprotein levels but otherwise no signs of malignant B cell disease after at least 3 years of follow-up Because abnormalities in cellular immunity are probably slight in these patients sensitive methods have to be applied We therefore studied the response to primary immunogens namely skin reactivity to dinitrochlorobenzene (DNCB) measured semiquantitatively (1) and in vitro lymphocyte transformation by haemocyanin of Helix pomatia (HPH) following immunization (8 19) In vitro lymphocyte transformation by antigens can be used as a parameter of cellular immunity as sensitized T cells are needed to initiate the reaction (9)

In addition in vitro lymphocyte transformation by phytohaemagglutinin (PHA) pokeweed mitogen (PWM) and a cocktail of secondary antigens was measured as well as the influence of autologous serum on lymphocyte transformation

STUDY POPULATION AND METHODS

Paraproteins were identified by immunoelectrophoresis with *monospecific antisera against IgG IgA IgM and* kappa and lambda light chains Ig were quantified with radial immunodiffusion using Tri Partigen plates (Behring werke)

Table I gives clinical details of the 20 patients Group P_1 comprises the patients with high serum paraprotein levels P_2 the patients with benign paraproteinaemia on the basis of low serum paraprotein levels almost normal other Ig levels and low plasma cell (or atypical lymphocyte) percentage in the bone marrow (sternal puncture) All patients were in good general health Serum paraprotein levels were stable during the follow up Skeletal X ray studies were normal at the time of the study In all pa

Table I *Clinical data on the patients*

Pat no	Para-protein	Age (y)	Sex	Immunoglobulins (mg/100 ml) IgG	IgM	IgA	Total serum protein (g/100 ml)	Blood picture Hb (g/100 ml)	Leuc (×1000/mm^3)	Thromb (×1000/mm^3)	Plasma cells or atyp lymph in bone marrow (%)	Follow up before study (y)	Other diseases
Group P_1													
1	IgGλ	67	♀	3 895	108	73	8 0	14 8	6 5	185	10	3	Cholecystector
2	IgGκ +IgAλ	64	♂	3 977	114	894	8 4	13 0	6 1	168	4	4	
3	IgGλ	62	♂	3 488	34	56	7 6	12 6	6 4	208	7	7	
4	IgGλ	69	♂	4 613	114	73	8 8	16 7	6 2	216	0 5	6	Chron bronch
5	IgGλ	65	♂	4 387	91	222	7 7	12 3	4 1	182	3	5	Chron bronchit
6	IgMλ	42	♂	780	3 003	112	7 5	15 6	7 6	155	9	3	
7	IgMκ	66	♂	847	2 562	60	7 3	14 3	4 5	163	5	5	
8	IgAλ	72	♀	946	18	1 848	7 2	11 7	6 5	206	10	3	Temp epilepsy
Group P_2													
9	IgGλ	50	♂	2 253	198	142	7 7	15 1	6 4	182	3	6	
10	IgGκ	51	♂	1 544	34	134	6 7	14 1	9 2	217	0 2	4	
11	IgGλ	50	♂	2 415	66	108	8 0	13 2	4 5	208	2	5	
12	IgGλ	58	♂	1 558	80	110	7 7	16 8	11 6	178	2	3	Angina pector
13	IgGλ	69	♂	1 749	115	165	7 2	15 5	7 9	197	2	3	Myoc infarctio
14	IgGλ	59	♂	1 683	224	262	7 2	15 0	6 2	155	1	3	
15	IgGκ	70	♂	1 353	550	124	7 2	17 6	6 4	134	0 5	3	
16	IgGκ	52	♀	1 638	154	119	7 6	13 8	5 0	194	2	3	Recurrent cystit
17	IgMλ	52	♂	979	453	97	6 3	13 3	7 7	218	10	4	
18	IgMκ	66	♂	891	950	67	7 0	16 0	7 2	278	9	3	Chron bronchit
19	IgAλ	57	♂	803	50	1 380	7 4	15 4	5 1	159	1	6	
20	IgAλ	63	♂	869	176	940	7 5	17 8	5 7	194	1	3	Kidney stone+ infection

ents Bence Jones protein in 50 times concentrated urine (Minicon® B_{15} Amicon USA) was absent according to immunoelectrophoresis using antisera specific against free kappa and lambda light chains The patients used no drugs during the study and had never been on corticosteroid or cytostatic therapy

Eighteen controls (2 females) in the same age range (4?–73 years, mean 59) with various complaints were visiting the Outpatient Clinic In 10 no abnormalities could be discerned in 2 untreated coronary heart disease was present in 3 uncomplicated hypertension in 2 untreated chronic bronchitis and 1 had had a cholecystectomy 10 years ago

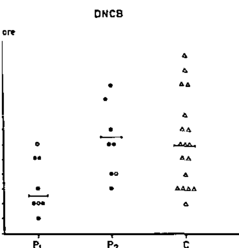
Patients and controls were studied simultaneously Three weeks after primary immunization with 1 mg HPH subcutaneously as previously described (8 19) blood samples were obtained for lymphocyte cultures Patients and controls gave informed consent

Lymphocyte cultures

Lymphocytes were isolated from defibrinated blood by Isopaque Ficoll gradient centrifugation 3 10^6 lymphocytes were cultured in Nunc tubes in 1 ml MEM Eagle with 20% pooled human serum of male blood donors and with 20% autologous serum Serum was inactivated at 56 C for 30 min and supplemented with penicillin 100 IU/ml and streptomycin 100 μg/ml as described by Du Bois et al (7) The cultures with PHA (Burroughs Wellcome 50 5 and 1 μl/ml were incubated for 3 days at 37°C wi 5% CO_2 in air while cultures without stimulant wi HPH 15 μg/ml antigen cocktail (containing PPD 10 μ ml candida antigen purified extract 15 μg/ml and mump vaccin from Ely Lilly 50 μl/ml 1 500 diluted) and PW (Grand Islands Biological Cie) 10 μg/ml were incubate in the same way for 6 days Twenty four hours befor harvesting 0 5 μCi ^{3}H thymidine (Radiochemical Centr Amersham specific activity 40 mCi/mmol) was added Cultures were harvested by filtration through fiber glas filters The radioactivity of the dried filters was counte in a Packard liquid scintillation counter and expressed disintegrations per minute (dpm) per culture The culture were carried out in triplicate The arithmetical mean wa calculated The viability of the cultures after 6 days wa checked morphologically The highest response of th three PHA doses was taken Antigen induced lymphocyt transformation was expressed as stimulation ratio be tween the culture stimulated with the antigen and th parallel culture without antigen

$$\frac{\text{dpm/culture with antigen}}{\text{dpm/blank culture}}$$

A ratio of <2 was considered as negative lymphocyte transformation 2–10 as slight and >10 as good



ᵢ 1 DNCB skin reactivity (semiquantitative score) in ᵢups P_1 (high paraprotein levels) and P_2 (low levels) and ntrols (C Δ) Cross bars=median values ●=IgG para =IgM para ◐=IgA para

ᵢCB score

ᴵCB skin reactivity was measured semiquantitatively E Bleumink and J P Nater from the Department of rmatology University Hospital Groningen as previ ly described (1) In short for sensitization 2 mg of ᴵCB in acetone (British Drughouse recrystalized twice) brought on the volar side of the right underarm in a lythene ring (diameter 2 cm) After 14 days challenges re done by patch testing (Scandinavian silverpatch olle Lassen Denmark) with 30 10 and 3 μg of DNCB in l ml volume on the skin of the back After 48 hours the action was measured as follows 1+ erythema 2+ ery ema and induration 3+ as before and vesiculation 4+ d ulceration The sum of reactions to the 3 doses yield the DNCB score

Statistical analysis was done with Wilcoxon s rank sum st

RESULTS

NCB skin reactivity

NCB scores of the patient groups P_1 and P_2 and ontrols are shown in Fig 1 DNCB scores in group ᵢ were significantly lower than in group P_2 ($p<$ 05) but not significantly lower than in controls o differences were found between the paraprotein lasses

IPH induced lymphocyte transformation

After primary immunization in vitro lymphocyte ransformation expressed as stimulation ratio was ignificantly lower in group P_1 (median 1 95) than in group P_2 (median 21 0 $p<0$ 02) and in controls (median 21 6 $p<0$ 02) (Fig 2) Four of five patients with high IgG paraprotein serum levels had no HPH induced lymphocyte stimulation (ratio <2) the fifth patient had slight stimulation whereas the two patients with high IgM paraprotein serum levels had good stimulation

Antigen cocktail induced lymphocyte transformation

Lymphocyte transformation by secondary antigens revealed good stimulation ratios in the majority of patients and controls (Fig 3) There were no differ ences between groups P_1 and P_2 and controls

PHA and PWM induced lymphocyte transformation

Low PHA results expressed as dpm values per cul ture were preferentially found in group P_1 (Fig 4) Differences between groups P_1 and P_2 and between group P_1 and controls were significant (both $p<$ 0 01) PWM induced lymphocyte transformation was similar in both patient groups and controls

Cultures in autologous serum medium did not show in any of the patients or controls an inhibit ing or stimulating effect upon PHA or antigen in duced lymphocyte transformation compared with the cultures in homologous serum

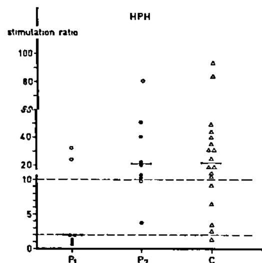


Fig 2 HPH induced in vitro lymphocyte transformation expressed as stimulation ratio (<2=no 2–10=slight >10=good stimulation) Abbreviations and symbols as in Fig 1

Table I. Whole blood ionized calcium (b Ca++), TmP, GFR, TmP/GFR and i PTH in 5 parathyroidectomized patients before (B) and after (A) 1 α OH D₃ treatment

Pat no	Sex	Age (y)	b-Ca+ (mmol/l) B	b-Ca+ (mmol/l) A	TmP (μmol/min) B	TmP (μmol/min) A	GFR (ml/min) B	GFR (ml/min) A	TmP/GFR (μmol/ml) B	TmP/GFR (μmol/ml) A	i PTH (ng/ml) B
1	♂	55	0.83	1.02	135.4	109.5	87.7	82.7	1.54	1.32	1.8
2	♀	30	0.74	0.98	87.8	67.2	64.0	57.0	1.37	1.18	1.8
3	♀	52	0.91	1.09	65.1	58.2	66.0	58.7	0.99	0.99	1.6
4	♀	50	0.73	1.01	59.6	50.3	64.9	63.7	0.92	0.79	1.7
5	♀	53	0.81	1.07	69.8	60.2	65.8	59.3	1.06	1.02	1.6
Mean		48	0.80	1.03	83.5	69.1	69.7	64.3	1.18	1.06	1.7

was investigated in a parathyroidectomized patient whether 1-α OH D₃ treatment would blunt the well known depression of TmP which can be induced by hyperglycemia (5, 10).

MATERIAL AND METHODS

The study population consists of 5 parathyroidectomized patients, 4 females and 1 male, with a mean age of 48 years (range 30–55).

Two patients (nos. 1 and 2) had well functioning kidney allografts with stable creatinine clearances of 85 and 60 ml/min 38 and 81 months after grafting. At the time of the present study patient 1 received 7.5 mg of prednisone and mg of azathioprine/day, while patient 2 had been out prednisone treatment for 15 months but still received azathioprine 100 mg/day. Patient 1 was totally parathyroidectomized 14 months after grafting (24 months prior to the present investigation) due to persisting hyperparathyroidism. Patient 2 was totally parathyroidectomized during hemodialysis treatment 5 months before grafting (86 months prior to the present investigation).

Three female patients (nos. 3, 4 and 5) with stable creatinine clearances of about 60 ml/min had been thyrodectomized 14, 6 and 23 years prior to the present investigation. During surgery they were accidentally parathyroidectomized and developed typical hypocalcemic tetany postoperatively.

In all 5 patients continued calciferol treatment was necessary to prevent development of symptomatic hypocalcemia and they were therefore considered to be totally parathyroidectomized. Apart from calciferol which was given to all 5 patients and the immunosuppressive treatment given to patients 1 and 2 they received no other medication at the time of the present study.

Patients 1–4 were normocalcemic on calciferol treatment. Calciferol was withdrawn and subsequently the patients were carefully followed by clinical observation as well as by weekly determination of ionized calcium in whole blood (b-Ca). They were all *moderately* hypocalcemic 14–72 days (mean 30) later with b-Ca between 0.73 and 0.91 mmol/l (normal range 1.02–1.18) but without manifest tetany.

Patient 5 was hypocalcemic due to deficient control. Also in this patient calciferol was withdrawn and at time of the present study her b-Ca++ was 0.81 mmol/l.

During *hypocalcemia* the TmP/GFR index was determined in two periods of 120 min in patients 1–4 and one period of 120 min in patient 5 according to the technique previously described (12, 13). Determination of TmP/GFR has been found to be reproducible with a coefficient of variation of 14% by duplicate measurements in 25 individuals. In the middle of each period plasma standard bicarbonate, b-Ca (Orion Model SS20 (I extracellular volume (calculated from ⁵¹Cr EDTA distribution space (9)) and i PTH (2) were determined in the patients.

The patients then received 1-α OH D₃ (Leo Pharmaceuticals, Copenhagen) orally in a dose varying between 1 and 4 μg/day and stable normocalcemia was thereby reestablished in the course of 14–27 days (mean 17.8).

During *normocalcemia* exactly the same investigations were carried out as during hypocalcemia. The 5 patients thus served as their own controls before and after administration of 1-α OH D₃.

To elucidate whether thyrotoxic patients exhibit TmP/GFR values in excess of those obtained in parathyroidectomized patients, TmP/GFR was measured in two thyrotoxic patients, one female and one male 67 and 68 years of age with creatinine clearances of 85 and ml/min respectively.

Finally the decrease in TmP induced by hyperglycemia was studied. In one parathyroidectomized patient (no. TmP/GFR was determined in one 120 min period with normoglycemia and in one period with hyperglycemia before as well as after administration of 1-α OH D₃. Hyperglycemia was induced by i.v. injection of 50 ml 50% glucose at time zero and at time 60 min during the second 120 min period. The total dose of glucose administered was 278 mmoles. Informed consent was obtained from the patients.

RESULTS

Hypoparathyroid patients

Table I shows the values of b Ca+, TmP/GFR and i PTH before and after treatment with 1 α OH D

The patients were hypocalcemic before trea

Table II *TmP/GFR in a totally parathyroidectomized patient (no 5) during normo- (N) and hyperglycemia (H) before (B) and after (A) treatment with 1 α OH D_3*

	Plasma glucose (mmol/l)		Diuresis (ml/120 min)		b-Ca ⁺ (mmol/l)	TmP/GFR (μmol/ml)		i PTH (ng/ml)
	N	H	N	H		N	H	
B	5 8	19 0	650	235	0 81	1 06	0 62	1 6
A	4 7	17 1	320	235	1 07	1 02	0 73	1 6

Maximal value

ment due to withdrawal of calciferol (patients 1–4) or due to deficient control of calciferol dosage (patient 5) The mean b-Ca^{++} during hypocalcemia was 0 80 mmol/l (range 0 73–0 91)

Stable normocalcemia was established following 14–27 (mean 17 8) days of treatment with 1 α-OH D_3 and b Ca^{++} increased by 0 23 mmol/l (range 0 18–0 28) The mean b Ca^{++} during normocalcemia was 1 03 mmol/l (range 0 98–1 09)

Serum i PTH was unchanged at low values (mean 1 7 ng/ml) before as well as after treatment with 1-α-OH D_3

The TmP/GFR remained unchanged in one patient (no 3) while a small but insignificant ($p>0$ 05) decrease was observed in 4 patients (nos 1 2 4 and 5) after treatment with 1-α OH D_3 The mean decrease in TmP/GFR was only 0 12 μmol/ml and was uncorrelated ($p>0$ 05) to the increase in b-Ca^{++}

Standard bicarbonate was identical before (mean 23 2 mmol/l range 21 7–24 5) and after (mean 24 1 mmol/l range 21 4–25 5) treatment with 1 α OH D_3 The extracellular volume likewise remained unchanged before (mean 12 2 l range 10 7–13 8) and after (mean 11 5 l range 9 5–13 6) treatment

Hyperthyroid patients

The TmP/GFR was 1 54 and 1 56 μmol/ml respectively in the two hyperthyroid patients and thus 45 8% and 47 5% higher than the mean of all values in the hypoparathyroid patients The i PTH values of these 2 patients were within the normal range (1 7 and 1 8 ng/ml respectively)

TmP/GFR during normo- and hyperglycemia

TmP/GFR was measured during normoglycemia and during hyperglycemia before as well as after treatment with 1-α OH D_3 It appears from Table II that hyperglycemia results in a considerable depression (41 5%) of TmP/GFR and that treatment with *1-α OH D_3* does not counteract this depression

DISCUSSION

In a previous study (13) on patients with intact parathyroid glands it was demonstrated that treatment with *1-α-OH D_3 was followed by a significant* increase in TmP/GFR and a significant decrease in i PTH Taking into account all values before as well as after treatment with 1-α OH D_3 a significant inverse correlation was found between TmP/GFR and i PTH Based on these results it was suggested that changes in the renal handling of phosphate induced by 1-α OH D_3 might be explained as being mediated via the concomitant suppression of PTH A direct stimulation by biologically active vitamin D on the tubular reabsorption of phosphate could however not be completely excluded for which reason the present investigation on totally parathyroidectomized patients was carried out

The i PTH values of the parathyroidectomized patients were low and in contrast to patients with intact parathyroid glands unaffected by administration of 1-α-OH D_3 The i PTH values were however not below the normal range in these obviously hypoparathyroid patients reflecting the well known limitations of bovine PTH assays at low hormone concentrations (3)

The TmP/GFR ratios of the parathyroidectomized patients were higher than in patients with preserved parathyroid glands (13) This finding is in good agreement with the previously demonstrated (12) inverse correlation between TmP/GFR and i PTH Furthermore in no case did the TmP/GFR ratio increase after treatment with 1-α OH D_3 and the 5 hypoparathyroid patients thus responded in a manner which differs distinctly from patients with intact parathyroid glands (13) In fact

a small but insignificant decrease in the ratio was observed in 4 of the 5 hypoparathyroid patients

By itself this finding does not prove that 1-α-OH D_3 is without stimulating effect on tubular phosphate reabsorption The TmP/GFR in parathyroidectomized man may be maximal in the sense that no further increase is possible by any stimulus including biologically active vitamin D In agreement with Bijvoet (4) we have however found that thyrotoxic patients may exhibit TmP/GFR ratios considerably in excess of the ratios in hypoparathyroid patients indicating that TmP/GFR is not maximal in hypoparathyroidism Furthermore the results shown in Table II demonstrate that 1-α-OH-D_3 does not counteract the depression of TmP/GFR which can be induced by hyperglycemia.

Based on all the presented evidence the present investigation suggests that biologically active vitamin D does not stimulate tubular phosphate reabsorption and that the previously demonstrated antiphosphaturic effect of 1-α-OH D_3 is mediated largely if not exclusively via a concomitant suppression of PTH

This conclusion is in accordance with the results of several investigations on normal and para rats (6 8 17) most recently con by Popovtzer et al (16) who reported that 25-hydroxycholecalciferol reduces urinary phosphate excretion only in normal but not in parathyroidectomized rats

Our findings on man as well as the above mentioned findings on rats are however at variance with the studies of Puschett et al (18) who found that active vitamin D increases tubular phosphate reabsorption in volume expanded parathyroidectomized dogs Species differences as well as differences in experimental design may account for this discrepancy

Further work is necessary to finally settle the problem regarding the effect of vitamin D on the renal handling of phosphate Apart from micropuncture studies further information may be obtained from investigations on patients under conditions where the effect of vitamin D PTH a calcium can be dissociated

ACKNOWLEDGEMENTS

This study was supported by grants from P C Petersens Fond and Fonden til Lægevidenskabens Fre me

REFERENCES

1 Agus Z S Gardner L B Beck L H & Goldbe M Am J Physiol 224 1143 1973
2 Almqvist S Hjern B & Wasthed B Acta E docrinol (Kbh) 78 493 1975
3 Berson S A & Yalow R S Am J Med 50 6 1971
4 Bijvoet O L M Clin Sci 37 23 1969
5 Cohen J J Berglund F & Lotspeich W D Am Physiol 180 156 1955
6 Crawford J D Gribetz D & Talbot N B Am Physiol 180 156 1955
7 Harrison H E & Harrison H C J Clin Inve 20 47 1941
8 — Metabolism 13 952 1964
9 Ladefoged J Eur J Clin Invest 5 72 1975
10 Levitan B A J Appl Physiol 4 224 1951
11 Madsen S & Ølgaard K Clin Chem Subm 1976
12 Madsen S Ølgaard K & Ladefoged J Acta M Scand 200 7 1976
13 — Acta Med Scand 200 351 1976
14 Massry S G Coburn J W Fnedler R M Kurokawa K & Singer F R Nephron 15 1975
15 Massry S G Fnedler R M & Coburn J W Ar Intern Med 131 828 1973
16 Popovtzer M M Robinette J B Deluca H F Holick M F J Clin Invest 53 913 1974
17 Puschett J B Beck W S & Jelonek A Scien 190 473 1975
18 Puschett J B Moranz J & Kurnick W S J Cl Invest 51 373 1972
19 Slatopolsky E Robson A M Elkan I & Bri N S J Clin Invest 47 1865 1968
20 Stamp T C B & Stacey T E Clin Sci 39 1970
21 Zerwekh J E Brumbaugh P F Haussler D H Cork D J & Haussler M R Biochemistry 13 1974

Acta Med Scand 202 27–32 1977

Aseptic Necrosis of Bone Following Renal Transplantation

Clinical and Biochemical Aspects and Bone Morphometry

H E Nielsen F Melsen and M S Christensen

From First Medical University Clinic Århus Kommunehospital University Institute of Pathology Århus Amtssygehus and the Institute of Pharmacology University of Århus Århus Denmark

ABSTRACT Aseptic necrosis of bone has developed 5–48 months (mean 23) after renal transplantation in 22 (11%) of 195 patients with graft function for more than 6 months The bone lesions were mainly localized to weight bearing cancellous bone areas, most often the femoral heads The mean duration of hemodialysis was significantly longer in recipients with aseptic necrosis than in those without The serum concentration of phosphate was reduced and the serum concentration of parathyroid hormone increased in both groups of recipients Quantitative histological examination of iliac crest biopsies showed a pronounced reduction of spongy bone (osteopenia) in recipients with aseptic necrosis compared with both normals and recipients without aseptic necrosis Both patient groups showed similar changes in bone remodelling, indicating decreased bone formation These findings suggest that osteopenia is an important contributory factor in the development of aseptic necrosis of bone after transplantation The osteopenia may be a consequence of both the uremic bone disease before transplantation and the immunosuppressive treatment after transplantation

Aseptic necrosis of bone especially in weight bearing regions is a frequent complication to renal allotransplantation (4 9 24) However the pathogenesis is not established Steroid induced osteoporosis (10 25) ischemia of bone due to fat embolism or vascular changes of other etiology (11 16 26) persistent secondary hyperparathyroidism (1 5) as well as hypophosphatemic osteomalacia (4) have been suggested as pathogenetic factors

The present investigation was performed in an attempt to clarify the etiology of posttransplant aseptic necrosis by measurement of biochemical indices of calcium metabolism including serum parathyroid hormone (PTH) and quantitative histological evaluation of iliac crest biopsies

PATIENTS

During the years 1964–74 291 patients (9–65 years of age) received kidney allografts and 195 of them had graft function 6 months after transplantation (long term survivors) Immunosuppressive therapy with prednisone and azathioprine was given with only minor changes in dosage schedule over the years The daily dose of prednisone in uncomplicated cases was about 25 mg 3 months after 10–15 mg 12 and 5–10 mg 24 months after transplantation The dose of prednisone given during the first 3 months after transplantation was calculated for the 20 patients from whom a bone biopsy was taken The usual daily dose of azathioprine was 2 mg/kg b wt Aluminium containing antacids were given in the first 3–4 weeks following transplantation

METHODS

The fasting serum concentrations of calcium inorganic phosphate and alkaline phosphatase were measured by autoanalyzer methods every 3 months Serum calcium was corrected for individual variations in serum protein concentration and calculated as the calcium concentration corresponding to a protein level of 7 g/100 ml serum (23) The serum concentration of PTH was measured on extracts of serum by a sensitive radioimmunoassay (6)

Radiological examination of the skeleton was carried out before transplantation and afterwards at intervals of 1–2 years

After double tetracycline labeling a transiliac bone biopsy (3) was taken from 13 long term survivors with radiologically progressive bone necrosis and 7 recipients without aseptic necrosis The 7 control patients were selected to match those with osteonecrosis as regards the mean time since transplantation graft function age and sex Bone morphometric values from 34 normal individuals with the same age and sex distribution as the long term survivors served as normal control values (19) Using simple measurements and the poi

Table I *Clinical data on the 22 long term survivors developing aseptic necrosis of bone*

Pat no	Age (y)	Sex	Duration of dialysis (mo)	Renal disease	Onset of aseptic necrosis (mpt)	Hyper calcemia (mpt)	Hypophos phatemia (mpt)	Duration of immuno- suppressive therapy (mo)
20	24	♀	1	MKD	13	0–17	3– 17	114
26	24	♂	2	CPN	51		0– 5	108
52	18	♀	20	MKD	48			81
57	28	♂	4	CRD	38		52– 96	89
63	18	♀	2	CGN	12		42–104	86
72	28	♂	9	CGN	31		29– 40	82
90	51	♂	28	CGN	14	?–73 (42)	0– 73	73
92	39	♂	7	AN	34			70
96	58	♂	4	AN	8	?–70 (60)	0– 70	70
102	52	♀	1	PKD	10			69
119	34	♀	1	U	23			67
127	31	♂	18	CGN	19		0– 8	55
132	23	♂	7	CGN	18	3–8		60
136	44	♂	10	CGN	50		13– 25	58
141	24	♂	1	CGN	15		0– 26	58
146	29	♂	19	CGN	5			57
152	30	♀	4	CRD	22			60
169	24	♀	24	MKD	10			57
170	25	♂	13	CGN	14	6–16	0– 30	40
199	35	♀	0	AN	34			48
226	25	♀	18	U	5			35
248	47	♂	0	AN	27			31
Mean	32 3		8 8		22 8			66 7
S D	11 4		8 7		14 4			21 0

mpt=months post transplantation MKD=medullary kidney disease CPN=chronic pyelonephritis CRD=congen[ital] renal disease CGN=chronic glomerulonephritis AN=analgesic nephropathy PKD=polycystic kidney disease U=[un]known

following measurements were done on undecalcified [?] μm thick sections embedded in methylmethacrylate (21) *absolute volume of trabecular bone (AVTB)* as the percentage of the sections occupied by trabecular bone *osteoid surfaces (OS)* in per cent of total trabecular bone surfaces *relative osteoid volume (OV)* in per cent of total trabecular bone volume *mean width of osteoid seams (WOS)* in μm as the mean of four extreme measurements in all surfaces covered with osteoid *trabecular osteoclastic resorption surfaces (RS)* in per cent of total trabecular bone surfaces The mineralization activity was evaluated in ultraviolet light on 20 μm thick undecalcified unstained sections by determining (15) *calcification rate in trabecular bone (CR)* in μm per day as the mean distance between the middle of the fluorescent tetracycline lines in all double labeled zones and the *active trabecular calcification surfaces (ATCS)* as the tetracycline labeled surfaces in per cent of total trabecular bone surfaces as described in detail elsewhere (20)

Mann Whitney rank sum test or Student s *t* test were used for comparison of group means Spearman s rank correlation test for correlation analysis and the χ^2 test for comparison of frequencies in groups

RESULTS

Frequency Radiologically manifest aseptic necrosis of bone developed in 22 of the 195 long term survivors (Table I) In patients more th[an] 40 years old the frequency was 5 8% compar[ed] with 15 6% in patients below 40 years of a[ge] (p<0 05) There was a significantly higher fr[e]quency (19 0%) (p<0 05) in patients dialysed f[or] more than 6 months than in patients dialysed f[or] shorter periods (8 0%) No correlation was fou[nd] between the frequency of osteonecrosis and se[x] primary renal disease or source of the kidney (livi[ng] donor or cadaver kidney) The patients with asep[tic]

Table II *Localization of aseptic necrosis of bo[ne]*

	Affection	No o[f] pats
Head of femur	Bilateral	11
	Unilateral	7
Condyles of femur	Bilateral	4
	Unilateral	2
Ankle bone	Unilateral	[illegible]
Head of humerus	Bilateral	3
	Unilateral	1
Capitulum of humerus	Unilateral	[illegible]



Fig 1 Aseptic necrosis of the left femoral head (*left*) and the right femoral head without bone affection (*right*) 77 months after renal transplantation Patient 26

tic necrosis had not received greater doses of prednisone during the first 3 months after transplantation than the patients without bone lesions

Onset and localization The radiological signs of aseptic bone necrosis were first detected 5–48 months (mean 23) after renal transplantation The patients had suffered from pains and decreased mobility in the affected bones and joints for 0–11 months before the X ray diagnosis The radiological findings are given in Table II Characteristically the bone necrosis was present in weight bearing bones composed mainly of cancellous bone and most frequently localized to the femoral heads (Fig 1) often with bilateral affection Both osteonecrosis and spontaneous fractures were found in 2 patients No correlation was found between the side localization of the graft and the unilateral necrosis of the femoral head At the time of this

Table III *Biochemical values (mean ±S D) in renal transplanted (RT) patients with and without aseptic necrosis of bone (ON) and in normal controls*

	S-calcium corr prot (mg/100 ml)	S phosphate (mg/100 ml)	S PTH (pg/ml)	S-creatinine (mg/100 ml)
RT patients				
With ON ($n-17$)	9 90±0 41	2 95±0 40	92±3?	1 15±0 26
Without ON ($n=58$)	9 96±0 37	? 96±0 65	115±49	1 25±0 35
Controls ($n-64$)	9.86+0 31	3 57±0 56	67±18	0 90±0 1

Corrected for individual variations in S protein concentral

Table IV *Bone morphometry (mean±S E M) in renal transplanted (RT) patients with and without aseptic necrosis of bone (ON) and in normal controls*

Abbreviations of bone morphometry are explained under Methods

	AVTB	OS	OV	WOS	RS	CR	ATCS
RT patients							
With ON (n=10)	11 2±1 1	32 0±3 7	4.8±1 0	8 2±0 7	4 2±0 5	0.53±0 03	17.5±3.2
Without ON (n=7)	19 1±1 0	24 5±3 2	2 3±0 7	7 9±0 8	4 5±0 8	0 50±0 03	9 8±2 1
Controls (n=34)	21 8±1 2	15 7±1 6	2 0±0 3	9 0±0 3	3 9±0 2	0 65±0 03[a]	13.5±1.3[b]

[a] n=6 [b] n=15

study X rays showed progressive bone lesions in 14, partial or complete healing of the lesions in 5 and unchanging lesions in 3 patients

Biochemical measurements At the time of this study the mean serum concentration of phosphate was lower (p<0 001) and of PTH higher (p<0 001) in the long term survivors with and without aseptic bone necrosis than in the normal controls (Table III) No significant differences were found in the mean serum calcium concentrations The frequency of hypercalcemia was the same in patients with and without osteonecrosis A slight increase in serum creatinine was found in the total group of transplanted patients (p<0 001)

Bone morphometry Table IV gives the histo ıometric data in 17 renal transplanted pa with good graft function (creatinine clearance ≥50 ml/min) and in 34 normal controls The AVTB in iliac crest biopsies was markedly decreased (p<0 01) in long term survivors with aseptic necrosis compared with both normals and long term survivors without aseptic necrosis Both groups of patients showed a significant increase in the percentage of OS but the OV was increased only in patients with aseptic necrosis An equal decrease in CR (p<0 01) was found in both pa tient groups whereas the percentage of tetra cycline labeled ATCS the WOS and the RS were normal

An inverse but insignificant correlation was found between serum phosphate and OS (n=17 r=−0 46, 0 10>p>0 05) and a positive correlation between PTH and RS (n=17 r=0 46 0 10>p> 0 05)

In 4 of the 22 patients with osteonecrosis the graft function was reduced (creatinine clearance <50 ml/min) and s PTH moderately increased Bone biopsy from 3 of these patients showed osteomalacic renal osteodystrophy and osteopenia

DISCUSSION

The frequency of aseptic bone necrosis after renal transplantation has varied between 5 and 37% in previous studies (4 9 24) In the present study 11% of kidney recipients with a graft functioning for more than 6 months developed aseptic necrosis of bone most frequently localized to weight bearing areas consisting of cancellous bone i e the femoral head knee or ankle

The pathogenesis of aseptic necrosis of bone is not known The degree of bone changes present before transplantation and the persistence of renal osteodystrophy after transplantation (1 5) ischemia of bone (11, 26) hypophosphatemic osteomalacia (4) or steroid induced osteoporosis (10 25) have all been held responsible for the bone lesions

The significance of the severity of renal osteodystrophy before transplantation for the development of aseptic necrosis of bone after transplantation is not known This may be because the method most often used to evaluate bone structure i e radiology is not quantitative and because bone histomorphometry has not been available for large scale clinical use Renal osteodystrophy per se can however lead to osteonecrosis since this bone lesion has been reported in patients on chronic hemodialysis (2) None of the present patients developed aseptic necrosis of bone while on dialysis but there was an increased frequency of this bone lesion after transplantation in patients who had been dialysed for more than 6 months The bone morphometry in the patients with aseptic necrosis did not demonstrate persisting severe renal osteodystrophy in accordance with the very modest increase in serum PTH found in patients with as well as without osteonecrosis The frequency of aseptic necrosis in patients who devel-

ped hypercalcemia after renal transplantation did ot differ from that found in patients without hyper alcemia (7) However a remarkably high fre uency of osteonecrosis was recently reported n patients with post transplantation hypercalce nia (5)

Bone ischemia caused by fat embolism due to ver steatosis has been suggested to be a factor in he development of aseptic necrosis of the femoral ead in steroid treated patients (11 12) and in abbits (13) This pathogenesis of aseptic bone ecrosis is not probable in our patients among whom only one showed evidence of hepatic disease hepatic cirrhosis) Ischemia of the femoral head has been suggested (26) because a correlation was ound between the side localization of the graft and affected femoral head in unilateral necrosis of head of femur Since no such correlation was ound in our patients we cannot confirm this ex planation of etiology

Hypophosphatemic osteomalacia after renal transplantation has been described by Moorhead et al (22) In the present study serum phosphate was reduced to the same degree in long term sur vivors with and without aseptic necrosis Bone morphometry showed an inverse but insignificant correlation between the amount of unmineralized bone and serum phosphate

The importance of steroid therapy for the devel opment of aseptic necrosis of bone has been re ported in several studies (12 14 17) The fre quency of osteonecrosis after renal transplantation was reported to be markedly lower during a pe riod with low steroid dosage in the first 3 weeks after transplantation than during a period with a 3 fold dosage (14) The decrease in bone mineral content measured by photon absorptiometry during the first year after renal transplantation has been shown to be more pronounced during the first months after transplantation when the steroid doses are highest (18) In this study the recipients with aseptic necrosis of bone had not received higher doses of prednisone during the first 3 months after renal transplantation than the recipients without bone necrosis

The most striking abnormality in the bone morphometry in our patients was the marked reduc tion in the amount of cancellous bone (osteopenia) in patients with aseptic necrosis compared with both long term survivors without necrosis and nor mals Laurent et al (17) reported a decreased amount of trabecular bone in iliac crest biopsies from 25 adult patients with primary aseptic necrosis of the femoral head and in 10 corticosteroid treated patients with this bone lesion In both our patient groups the mineralization activity was re duced to the same extent since the calcification rate was decreased ($p<0\ 01$) and the percentage of ATCS was normal The significantly increased per centage of OS in spite of a normal percentage of ATCS indicates a reduced mineralization activity of the osteoid The WOS was normal in both pa tient groups Therefore the appositional rate of osteoid (bone formation) must be reduced to the same extent as the calcification rate The decreased bone mineralization activity and bone formation rate are probably secondary to the combined im- munosuppressive therapy with steroids and azathioprine

The bone changes especially the osteopenia present before transplantation were probably more severe in the patients who developed aseptic bone necrosis after transplantation since the rate of cal cification and bone formation was equally reduced in recipients with and without osteonecrosis This is supported by the longer duration of hemodialysis in the patients who developed aseptic necrosis of bone after transplantation than in those without this bone lesion

It was not unexpected that the long term surviv ing renal recipients showed osteopenia It was remarkable however that it was found especially in the recipients with aseptic bone necrosis It is well known that senile osteoporosis is not as sociated with the clinical and radiological syndrome of aseptic osteonecrosis The relationship found in this study between radiological aseptic necrosis of bone and the histological reduction of the amount of bone is in accordance with the hypothesis (10 17 25) that aseptic necrosis of bone may be secondary to microfractures in the osteopenic bone causing local impairment of blood supply to the bone and consequently bone collapse in cancellous bone

REFERENCES

1 Arfi S Moreau F Heuclin C Kreis H Paolaggi J B & Auguier L osteonecrose aseptique de la transplantation renale A propos de 29 cas Rev Rhum Mal Osteoartic 42 167 1975
2 Bailey G L Griffiths H J L Mocelin A J Gundy D H Hampers C L & Merrill J P Avascular necrosis of the femoral head in patients

on chronic hemodialysis Trans Am Soc Artif Intern Organs 18 401 1972

3 Bordier P Matrajt H Miravet L & Hioco D Mesure histologique de la masse et de la resorption des travées osseuses Pathol Biol 12 1238 1964

4 Briggs W A Hampers C L Merrill J P Hager E B Wilson R E Birtch A G & Murray J E Aseptic necrosis in the femur after renal transplantation Ann Surg 175 282 1972

5 Chatterjee S N Friedler R M Berne T V Oldham S B Singer F R & Massry S G Persistent hypercalcemia after successful renal transplantation Nephron 17 1 1976

6 Christensen M S A sensitive radioimmunoassay of parathyroid hormone in human serum using a specific extraction procedure Scand J Clin Lab Invest 36 313 1976

7 *Christensen M S Nielsen H E & Torring S* Hypercalcemia and parathyroid function after renal transplantation Acta Med Scand 201 35 1977

8 Courpront P Données histologiques quantitatives sur le vieillissement osseux humain Thése Lyon 1972

9 Cruess R L Blennerhassett J MacDonald F R MacLean L D & Dossetor J Aseptic necrosis following renal transplantation J Bone Joint Surg 50A 1577 1968

10 Editorial Corticosteroid induced bone collapse Br Med J 1 581 1972

11 Evarts C M & Phalen G S Osseous avascular necrosis associated with renal transplantation Clin Orthop 78 330 1971

12 Fisher D E & Bickel W H Corticosteroid induced avascular necrosis A clinical study of seventy seven patients J Bone Joint Surg 53 A 859 1971

13 Fisher D E Bickel W H Holley K E & Ellefson R D Corticosteroid induced aseptic necrosis II Experimental study Clin Orthop 84 200 1972

14 Freiberger R H & Swanson G E Aseptic necrosis of the femoral heads after high-dosage corticosteroid therapy NY State J Med 65 800 1965

15 Frost H M Tetracycline based histological analysis of bone remodelling Calcif Tissue Res 3 211 1969

16 Harrington K D Murray W R Kountz S L & Belzer F O Avascular necrosis of bone after renal transplantation *J Bone Joint Surg 53* 203 1971

17 Laurent J Meunier P Courpront P Edouard C Bernard J & Vignon G Recherches sur la pathogénie des nécroses aseptiques de la tete femorale Nouv Presse Méd 2 1755 1973

18 Lindsay R MacPherson S G Anderson J B & Smith D A The value of bone density measurements in predicting the risk of developing avascular necrosis following renal transplantation In Proceedings of the XIth European symposium on calcified tissues p 242 F A D L Publishing Co Copenhagen 1975

19 Melsen F Mosekilde L & Melsen B Quantitative and dynamic studies of normal bone To be published

20 *Melsen F & Nielsen H E Osteonecrosis following* renal transplantation A quantitative histological study on iliac bone Acta Pathol Microbiol Scand (A) In press 1977

21 Meunier P Vignon G Vauzelle J L & Zech P Etude histologique quantitative de la resorption osteoclastique dans les hyperparathyroidies primitives et secondaires Pathol Biol 17 927 1969

22 Moorhead J F Wills M R Ahmed K J Baillod R A Varghese Z & Tatler G L V Hypophosphataemic osteomalacia after cadaveric renal transplantation Lancet 1 694 1974

23 Pedersen K O Protein bound calcium in human serum Quantitative examination of binding and its variables by a molecular binding model and clinical chemical implications for measurement of ionized calcium Scand J Clin Lab Invest 30 321 1972

24 Pierides A M Simpson W Stainsby D Alvarez Ude F & Uldall P R Avascular necrosis of bone following renal transplantation Q J Med 44 459 1975

25 Solomon L Drug induced arthropathy and necrosis of the femoral head J Bone Joint Surg 55B 246 1973

26 Ølgaard K & Heerfordt J Femoral head necrosis in renal transplanted patients Evidence of a haemodynamic etiological factor Scand J Urol Nephrol 9 64 1975

Acta Med Scand 202 33–38 1977

Bone Composition and Parathyroid Function in Chronic Renal Failure

Lars Tougaard Egon Sørensen Merete Sanvig Christensen
Jens Brøchner Mortensen Paul Rødbro and Arne W S Sørensen

From the Departments of Clinical Physiology Nephrology and Clinical Chemistry Ålborg Hospital South Ålborg and the Institute of Pharmacology University of Århus Århus Denmark

ABSTRACT The development of bone abnormalities has been studied in 24 patients with severe chronic renal failure The glomerular filtration rate (GFR) was between 5 and 25 ml/min The mean values of plasma calcium, degree of bone mineralization (P/Hypro) and bone mineral content (BMC) were subnormal, whereas the mean values of plasma phosphorus and serum parathyroid hormone (PTH) were elevated Analysis of the data revealed that the various parameters became increasingly pathological with decreasing renal function Serum PTH correlated inversely with both GFR and plasma calcium The decrease in bone P/Hypro with decreasing renal function could be explained by an inverse correlation to serum PTH Plasma alkaline phosphatase correlated inversely to both bone P/Hypro and BMC The present study on individual patients with varying degrees of renal insufficiency shows that the development of secondary hyperparathyroidism correlates with a reduction in the degree of bone P/Hypro and suggests that significant bone changes appear when the GFR falls below 15 ml/min

Bone disease and disturbed calcium metabolism are frequent findings in chronic renal failure (1 21) Prolongation of the life of uremic patients makes these complications increasingly important and accentuates the need for therapy To evaluate the effects of treatment methods that are suitable for assessing bone changes in clinical routine must be available Radiology is not quantitative and cannot show bone changes until gross abnormalities are present Morphometric analysis of bone can be evaluated only by experts and requires large biopsies Two other methods have however been developed from which quantitative information on bone composition can be obtained with little inconvenience to the patient the *degree of bone mineralization* can be estimated by measuring the phosphorus/hydroxyproline ratio in small bone biopsies (22), the *bone mineral content* can be estimated by photon absorptiometry in the forearm (9)

We have studied the development of bone abnormalities in chronic renal failure by measuring the degree of bone mineralization and the bone mineral content together with serum parathyroid hormone and other biochemical indices of calcium metabolism

PATIENTS

Twenty four patients (15 females and 9 males) aged 20–70 years (mean 51) with severe chronic renal failure were selected from a large nephrologic out patient clinic and studied if glomerular filtration rate (GFR) was between 5 and 25 ml/min and relatively stable Seventeen patients had chronic pyelonephritis four polycystic kidney disease two chronic glomerulonephritis and one renal hypoplasia None were on hemodialysis or undergoing treatment for bone disease All were on a normal diet None received phosphate binders anticonvulsants or hormones

METHODS

The patients were investigated while supine and after an overnight fast Plasma concentrations of calcium inorganic phosphorus and alkaline phosphatase were measured on a Technicon SMA® 12/60 system The serum concentration of parathyroid hormone (PTH) was measured by radioimmunoassay on extracts of serum using AS 211/32 and bovine parathyroid hormone for ^{125}I labeling and standard (7) GFR was measured from the total [^{51}Cr] EDTA plasma clearance determined by a simplified single injection method (3 5) The bone mineral content (BMC)

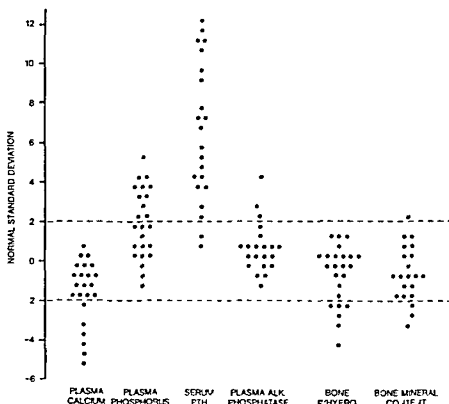


Fig 1 Individual values of plasma and bone indices in 24 patients with chronic renal failure The values are normalized with normal mean−0 00 and the normal S D as unit =normal reference values

in the forearm was measured by photon absorptiometry (9) The degree of mineralization was measured as the bone phosphorus/hydroxyproline ratio (P/Hypro) and based on double determinations (coefficient of variation ?%) of this ratio in a bone biopsy obtained from the iliac crest under local anaesthesia (22) Since the bone P/Hypro changes with age (23) and BMC with age and sex (8) these bone indices were expressed in % of the corresponding normal mean

Statistical analysis

Values of serum PTH and plasma alkaline phosphatase were logarithmically transformed Normal values were either the reference values of our Department of Clinical Chemistry or have been published elsewhere (7 8 23) Student s *t* test or the Mann Whitney *U* test were used to compare mean values of the patients with the normal values Analysis of correlation was performed with either two or three variables (13) Thus $r_{PTH\,GFR}$ indicates the correlation coefficient between serum PTH and GFR and $r_{PTH\,Ca\,GFR}$ the partial correlation between serum PTH and plasma calcium excluding the effect of GFR

RESULTS

Fig 1 shows individual values of the patients Table I means and S D of patients and controls mean

Table I *Plasma and bone indices in 24 patients with chronic renal failure compared with reference values*

	Chronic renal failure		Reference values		Significance of difference
	Mean	S D	Mean	S D	
Plasma					
Calcium (mmol/l)	2 29	0 22	2 50	0 15	$p<0\ 001$
Phosphorus (mmol/l)	1 52	0 30	1 18	0 19	$p<0\ 001$
PTH (log pg/ml)	2 59	0 52	1 75	0 14	$p<0\ 001$
Alkaline phosphatase (log U/l)	1 57	0 20	1 49	0 14	n s
Bone					
Phosphorus/hydroxyproline (% of normal)	95 3	10 9	100 0	7 0	$p<0\ 01$
Mineral content (% of normal)	86 6	23 8	100 0	16 5	$p<0\ 01$

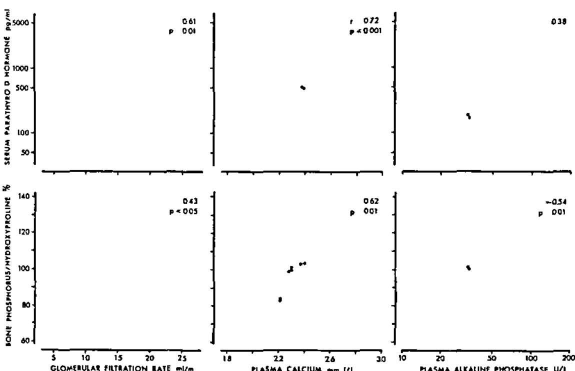


Fig 2 Serum parathyroid hormone and bone phosphorus/hydroxyproline ratio plotted against glomerular filtration rate plasma calcium and plasma alkaline phosphatase in 24 patients with chronic renal failure

plasma calcium was decreased and mean plasma phosphorus and mean serum PTH were increased Both the mean bone P/Hypro and the mean BMC were decreased Four patients had elevated plasma alkaline phosphatase but the mean value was not significantly increased

GFR was inversely correlated with plasma phosphorus ($r_{P\,GFR}=-0\ 41$ $p<0\ 05$) and positively correlated with plasma calcium ($r_{Ca\,GFR}=0\ 44$ $p<0\ 05$) The correlation between plasma calcium and plasma phosphorus was not significant ($r_{Ca\,P}=-0\ 38$ n s)

Serum PTH increased significantly with decreasing renal function and decreasing plasma calcium (Fig 2) Multiple correlation analysis showed that the variation in serum PTH was explained both by

Table II *Correlation coefficients between bone phosphorus/hydroxyproline (P/Hypro) bone mineral content (BMC) serum parathyroid hormone (PTH) and glomerular filtration rate (GFR) and biochemical indices of calcium metabolism in 24 patients with chronic renal failure*

	Bone P/Hypro		BMC		Serum PTH	
GFR	$r_{P/Hypro\,GFR}$ $r_{P/Hypro\,GFR\cdot PTH}$	= 0 43 0 01	$r_{BMC\,GFR}$	= 0 31	$r_{PTH\,GFR}$ $r_{PTH\,GFR\cdot C}$	= 0 61* = 0 47*
Plasma calcium	$r_{P/Hypro\,C}$ $r_{P/Hypro\,C\cdot PTH}$	0 62** − 0 05 n.s.	$r_{BMC\,C}$	= 0 19n	$r_{PTH\,Ca}$ $r_{PTH\,Ca\cdot GFR}$	=−0 72*** = 0 63**
Plasma phosphorus	$r_{P/Hypro\,P}$	=−0 12	$r_{BMC\,P}$	= 0 08	$r_{PTH\,P}$ $r_{PTH\,P\cdot C}$	= 0 48* = 0 32n.s.
Serum PTH	$r_{P/Hypro\,PTH}$	=−0 71 **	$r_{BMC\,PTH}$	=−0 22		
Plasma alkaline phosphatase (Ptase)	$r_{P/Hypro\,Ptase}$	= 0 54*	$r_{BMC\,Ptase}$	=−0 51**	$r_{PTH\,Ptase}$	= 0 38

n.s. no significance *0 05>p>0 01 0 01>p>0 001 **0 001>p

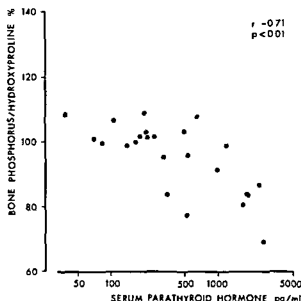


Fig. 3. Bone phosphorus/hydroxyproline ratio compared with serum parathyroid hormone in 24 patients with *chronic renal failure.*

decreased plasma calcium ($r_{PTH\,Ca\cdot GFR}$=0.63, $p<$ 0.01) and by the decrease in GFR ($r_{PTH\,GFR\cdot Ca}$= 0.47, $p<0.05$). The correlation between serum PTH and plasma phosphorus was not significant when the effect of plasma calcium was excluded ($r_{PTH\,P\cdot Ca}$=0.32, n.s.).

Bone P/Hypro decreased significantly with decreasing GFR and plasma calcium (Fig. 2), and correlated inversely with plasma alkaline phosphatase (Fig. 2) and serum PTH (Fig. 3). The mean bone P/Hypro for the 12 patients with serum PTH below 300 pg/ml was 103%, which is slightly supranormal ($p<0.05$). Multiple correlation analysis showed that neither GFR ($r_{P/Hypro\,GFR\cdot PTH}$=0.01, n.s.) nor plasma calcium ($r_{P/Hypro\,Ca\cdot PTH}$=0.23, n.s.) added significant explanation to the variation in bone P/Hypro that could not be explained by serum PTH alone. Bone P/Hypro and plasma phosphorus were not significantly correlated (Table II).

BMC was inversely correlated with plasma alkaline phosphatase but not with any other index studied (Table II), including bone P/Hypro ($r_{BMC\,P/Hypro}$ =0.35, n.s.).

DISCUSSION

In accordance with several earlier investigations (1, 11, 12, 21), the present study demonstrates marked disturbances in calcium metabolism in chronic renal failure. Furthermore, our results reveal that the degree of bone mineralization decreases with decreasing renal function, and that this change in bone composition could be explained solely by the secondary hyperparathyroidism.

The patients were selected from a large nephrologic outpatient clinic on the basis of their having a relatively stable renal function with GFR between 5 and 25 ml/min. Since this was the only criterion for entry into the investigation, the patients can be regarded as representative for (Danish) non-hemodialysed patients with chronic renal failure. Clinically manifest uremic bone disease was present in only one patient (GFR 5 ml/min), who had subnormal values of both BMC and bone P/Hypro together with elevation of alkaline phosphatase and PTH in blood.

Correlation analysis revealed that most of the indices studied were highly dependent on renal function. The significant correlations could be demonstrated partly because GFR was measured with high accuracy and precision (4, 5).

Elevation of serum PTH was the most frequent abnormality. In accordance with this finding, elevated serum PTH has previously been noted in patients with GFR below 40 ml/min (16). The high negative correlation coefficient which was found between serum PTH and bone P/Hypro demonstrates the relevance of secondary hyperparathyroidism for the development of bone changes in chronic renal failure. In this context it is noteworthy that a significant correlation has been found earlier between morphometric bone analysis and serum PTH (1).

The bone P/Hypro measures the proportion of mineral to collagen in bone, the degree of mineralization. This index depends on the etiology of bone changes. Low values of bone P/Hypro correlating with the reduction in serum 25-hydroxycholecalciferol have been found in, e.g., patients with vitamin D deficiency after partial gastrectomy (25). In the present study the bone P/Hypro correlated with GFR and plasma calcium and correlated inversely with both plasma alkaline phosphatase and serum PTH. Multiple correlation analysis showed that the decrease in bone P/Hypro in chronic renal failure could be explained solely by the secondary hyperparathyroidism. *Subnormal* values of bone P/Hypro were found in some patients in whom GFR was less than 10–15 ml/min and the serum PTH was highly increased. Low values of bone P/Hypro have been

found also in primary hyperparathyro dism (24) The morphological changes in primary hyperpara thyro d sm with increases in osteo d and peritra becular fibrosis will contribute to the low values of bone P/Hypro In a previous study (20) the mean bone P/Hypro was increased in the group of both und alysed and hemod alysed uremic patients be cause of h gh bone P/Hypro values in the d alysed pat ents The secondary hyperparathyroid sm is kno vn to decrease considerably during hemodial ys s due to the calc um supplements obtained from the d alysis bath (15) D fferent degrees of second ary hyperparathyro d sm n the two stud es probably expla n the difference in mean bone P/Hypro

Photon absorptiometry of the forearm estimates the bone mineral content with a h gh reproducib lity (10) and the BMC has been shown to correlate well with total body calc um measured by neutron acti vation analysis also in chron c renal failure (12) BMC was s gn ficantly inversely correlated with plasma alkal ne phosphatase but d d not correlate with any other index stud ed This may be due to the great interindiv dual variation in skeletal size reflected by the wide normal range of BMC 67–133% even after correction for variation due to age and sex (8) BMC measurements in ind v dual patients are therefore mainly of value to assess changes in BMC induced e g by therapy (10)

The correlat on between BMC and bone P/Hypro was not s gn ficant This was not unexpected as the nformat on obta ned by the two methods concerns different qual ties of the skeleton Photon absorptiometry on the forearm reflects changes in both spongeous and cortical bone (9) and the value is dependent on gross bone morphology Bone P/Hypro is determ ned in spongeous bone t ssue and th s index is in princ ple independent of the width and number of bone trabeculae

In recent years important new knowledge has been obtained concern ng v tamin D metabolism (14) It has been shown that the kidney is the sole s te of format on of 1 25-d hydroxycholecalciferol wh ch is h ghly active in increas ng the ntest nal ab sorpt on of calc um Low values of th s vitamin D metabol te have been reported in blood from uremic pat ents (17) The vitam n D res stance of urem c patients is in accordance with the hypothes s that decreased synthesis of 1 25-d hydroxycholecalci ferol n renal fa lure is respons ble for the decreased ntest nal calc um absorpt on the hypocalcaem a secondary hyperparathyro d sm and bone abnor malit es in renal fa lure Therapeut c trials of 1 25 d hydroxycholecalc ferol or the synthet c analogue 1α hydroxycholecalciferol have been undertaken in a few minor series (2 6 18 19 26) but the results so far have been inconclusive

The present study on individual patients with varying degree of renal insuff ciency has shown that the development of secondary hyperparathyro dism is correlated with a reduction in the degree of bone mineralization and suggests that sign f cant bone changes appear when the GFR falls below 15–10 ml/min

REFERENCES

1 Bord er P J Marie P J & Arnaud C D Evolu t on of renal osteodystrophy Correlat on of bone h stomorphometry and serum mineral and immunoreactive parathyro d hormone values before and after treatment with calc um carbonate or 25-hydroxy cholecalciferol K dney Int (Suppl) 2 102 1975

2 Brickman A S Sherrard D J Jowsey J S nger F R Baylink D J Maloney N Massry S G Norman A W & Coburn J W 1,25 Dihydroxy cholecalciferol Effect on skeletal les ons and plasma parathyro d hormone levels in uremic osteodystrophy Arch Intern Med 134 883 1974

3 Brøchner Mortensen J A simple method for the determination of glomerular f ltration rate Scand J Clin Lab In est 30 271 1972

4 Brøchner Mortensen J & Rødbro P Select on of routine method for determ nat on of glomerular f ltra t on rate n adult pat ents Scand J Clin Lab Invest 36 35 1976

5 — Comparison between total and renal plasma clear ance of [5] Cr EDTA S and J Clin Lab Invest 36 747 1976

6 Chan J C M Oldham S B Holick M F & De Luca H F 1-α Hydroxyvitamin D_3 n chron c renal fa lure A potent analogue of the kidney hormone 1 25-d hydroxycholecalc ferol J Am Med Assoc 234 47 1975

7 Christen en M S A sens t ve rad o mmunoassay of parathyro d hormone n human serum us ng a spec f c extraction procedure Scand J Cl n Lab Invest 36 313 1976

8 Christiansen C & Rødbro P Bone mineral content and estimated total body calc um n normal adults Scand J Cl n Lab Invest 35 443 1975

9 Christiansen C Rødbro P & Jensen H Bone mi neral content n the forearm measured by photon absorpt ometry Princ ples and reliab lity Scand J Cl n Lab In est 35 3 3 1975

10 Christiansen C Rødbro P & Lund M Effect of v tamin D on bone m neral mass in normal subjects and n ep lept c pat ents on anticonvulsants Br Med J 2 08 1973

11 Christiansen C Rødbro P & Mun k O Skeletal status n patients with chron c renal disease Scand J Urol Nephrol 10 70 1976

12. Cohn S H Ellis K J Caselnova R C Asad S N & Letteri J M Correlation of radial bone mineral content with total body calcium in chronic renal failure J Lab Clin Med 86 910 1975
13. Croxton F E Elementary statistics Dover Publications New York 1959
14. DeLuca H F The kidney as an endocrine organ involved in the function of vitamin D Am J Med 58 39 1975
15. Fournier A E Arnaud C D Johnson W J Taylor W F & Goldsmith R S Etiology of hyperparathyroidism and bone disease during chronic hemodialysis J Clin Invest 50 599 1971
16. Goldsmith R S Johnson W J & Arnaud C D The hyperparathyroidism of renal failure Pathophysiology and treatment J Clin Endocrinol Metab 3 305 1974
17. Mawer E B Backhouse J Taylor C M & Lumb G A Failure of formation of 1,25-dihydroxycholecalciferol in chronic renal insufficiency Lancet I 626 1973
18. Peacock M Gallagher J C & Nordin B E C Action of 1α hydroxyvitamin D_3 on calcium absorption and bone resorption in man Lancet I 385 1974
19. Pierides A M Ellis H A Simpson W Dewar J H Ward M K & Kerr D N S Variable response to long-term 1α-hydroxycholecalciferol haemodialysis osteodystrophy Lancet I 1092 19
20. Schaadt O & Tougaard L The degree of b mineralization in chronic renal failure estimated b phosphorus/hydroxyproline ratio in bone biops Acta Med Scand 198 91 1975
21. Stanbury S W Azotaemic renal osteodystroph J Clin Endocrinol Metab 1 267 1975
22. Tougaard L The degree of mineralization in b tissue The phosphorus/hydroxyproline ratio de mined on small amounts of bone tissue Scand J C Lab Invest 32 351 1973
23. — The degree of mineralization in normal bone ti estimated as the phosphorus to hydroxyproline ra Scand J Clin Lab Invest 35 285 1975
24. Tougaard L Hau C Rodbro P & Drzel J B mineralization and bone mineral content in prim hyperparathyroidism Acta Endocrinol 84 314 19
25. Tougaard L Rickers H Rodbro P Hess Thays E Christensen M S Lund B & Helmer Sorens O Bone composition and vitamin D after Pö gastrectomy Acta Med Scand 202 47 1977
26. Tougaard L Sorensen E Brochner Mortensen Christensen M S Rodbro P & Sorensen A W Controlled trial of 1α hydroxycholecalciferol in chr ic renal failure Lancet I 1044 1976

Acta Med Scand 202: 39–42, 1977

Bone, Calcium, and Hydroxyproline Metabolism in Hyperparathyroidism and after Removal of Parathyroid Adenoma

Ossi Laitinen

From the Second Department of Medicine, Helsinki University Central Hospital, Helsinki, Finland

ABSTRACT. Several laboratory parameters have been followed before and after surgery in a 69-year-old woman with parathyroid adenoma and severe skeletal involvement. High preoperative levels of immunoreactive parathyroid hormone rapidly decreased postoperatively, accompanied by a similar fall in the peptide-bound hydroxyproline (HP) excretion in the urine. Renal clearance of free HP was high both pre- and postoperatively, probably because of renal damage associated with the disease. The high serum alkaline phosphatase levels increased slightly after the operation. The patient developed severe postoperative hypocalcaemia, and prolonged calcium supplement therapy was necessary. The results imply a clear correlation between the changes in Ca homeostasis and the breakdown rate of bone collagenous matrix. Postoperative hypocalcaemia was mainly due to the rapid change in the rates of bone formation and destruction.

Hyperparathyroidism is characterized by increased bone turnover, particularly resorption, but findings suggesting that bone formation is also enhanced, at least in some cases, have been reported. However, several important aspects, such as the relative significance of bone involvement in the parathyroid hormone (PTH) regulated calcium (Ca) homeostasis, the possible interactions of the metabolisms of mineral proportion and organic matrix, as well as the more detailed metabolic background of skeletal changes in hyperparathyroidism, are still largely obscure (9).

In this study, the metabolism of bones has been analysed by assaying immunoreactive PTH, Ca in the urine and in the serum, urinary hydroxyproline (HP) and alkaline phosphatase (AFOS) prior to and after the removal of a parathyroid adenoma in a patient with severe skeletal changes. The clear changes in the parameters commonly used in the measurement of the activity of metabolic bone diseases may offer some new information on the nature of bone involvement in hyperparathyroidism.

CASE REPORT

A 69-year-old woman was sent to Helsinki University Central Hospital as a suspected case of Paget's disease. The suspicion had arisen on the basis of an increased serum Ca concentration and a greatly elevated HP excretion. Results of the more detailed laboratory analyses (6) were as follows: Ca in serum 12.4–14.0 mg/100 ml (reference value 9.0–11.0), Ca in urine 237–256 mg/24 h (50–220), total HP in urine 508–610 mg/24 h/m² (7–20), of which 252–306 mg (46–56%) in the form of free HP (in healthy subjects less than 5%, i.e. less than 1 mg/24 h/m² in this age group), serum HP 22.3–23.3 μg/ml (0.7–1.4), serum phosphorus 2.4–2.5 mg/100 ml (2.6–4.3), urinary phosphorus excretion 0.56–0.68 g/24 h (0.6–1.5), AFOS 630–720 U (normal less than 260). The disproportionally high serum Ca in relation to slightly elevated urinary Ca, the radiological findings of the severely osteoporotic bones, the histological examination of bone biopsy from iliac crest, and the highly abnormal ratio of free/total HP suggested that the patient suffered from hyperparathyroidism, a diagnosis which was later confirmed by the elevated level of immunoreactive PTH (measured in Stockholms Immunlaboratorium AB, Stockholm, by the method of Almqvist et al. (1)), 15.7 and 20.1 mg/ml in consecutive determinations.

Changes in four of the above-mentioned laboratory values after the removal of an adenoma weighing 7.45 g are recorded in Fig. 1. The PTH and total HP values displayed an abrupt and more or less simultaneous decrease. PTH concentration fell to normal on the 3rd day after operation. HP also decreased rapidly, but the output remained somewhat elevated (range 29.8–134.0 mg/24 h/m²)

12 Cohn S H Ellis K J Caselnova R C Asad S N & Letteri J M Correlation or radial bone mineral content with total body calcium in chronic renal failure J Lab Clin Med 86 910 1975
13 Croxton F E Elementary statistics Dover Publications New York 1959
14 DeLuca H F The kidney as an endocrine organ involved in the function of vitamin D Am J Med 58 39 1975
15 Fournier A E Arnaud C D Johnson W J Taylor W F & Goldsmith R S Etiology of hyperparathyroidism and bone disease during chronic hemodialysis J Clin Invest 50 599 1971
16 Goldsmith R S Johnson W J & Arnaud C D The hyperparathyroidism of renal failure Pathophysiology and treatment J Clin Endocrinol Metab 3 305 1974
17 Mawer E B Backhouse J Taylor C M & Lumb G A Failure of formation of 1 25-dihydroxycholecalciferol in chronic renal insufficiency Lancet 1 626 1973
18 Peacock M Gallagher J C & Nordin B E C Action of 1α hydroxyvitamin D_3 on calcium absorption and bone resorption in man Lancet 1 385 1974
19 Pierides A M Ellis H A Simpson W Dewar J H Ward M K & Kerr D N S Variable response to long term 1α hydroxycholecalciferol haemodialysis osteodystrophy Lancet 1 1092 1!
20 Schaadt O & Tougaard L The degree of bo mineralization in chronic renal failure estimated fro phosphorus/hydroxyproline ratio in bone biopsie Acta Med Scand 198 91 1975
21 Stanbury S W Azotaemic renal osteodystroph J Clin Endocrinol Metab 1 267 1975
22 Tougaard L The degree of mineralization in bo tissue The phosphorus/hydroxyproline ratio dete mined on small amounts of bone tissue Scand J Cl Lab Invest 32 351 1973
23 — The degree of mineralization in normal bone tiss estimated as the phosphorus to hydroxyproline rati Scand J Clin Lab Invest 35 285 1975
24 Tougaard L Hau C Rødbro P & Ditzel J Bo mineralization and bone mineral content in primar hyperparathyroidism Acta Endocrinol 84 314 197
25 Tougaard L Rickers H Rødbro P Hess Thayse E Christensen M S Lund B & Helmer Sørense O Bone composition and vitamin D after Poly gastrectomy Acta Med Scand 202 47 1977
26 Tougaard L Sørensen E Brochner Mortensen J Christensen M S Rødbro P & Sørensen A W S Controlled trial of 1α hydroxycholecalciferol in chro ic renal failure Lancet 1 1044 1976

hypocalcaemia after parathyroid surgery has become evident on the basis of the current series in this hospital (8) so far all the patients with definitely accelerated bone turnover (six cases with HP >50 mg/24 h/m², AFOS >260 U/l and detectable changes in bone X rays) have showed low postoperative Ca levels The lowest value has been 7 2 mg/100 ml in one and 5 1–6 4 mg/100 ml in five of these patients I v Ca infusions have been necessary in all patients some days to some weeks after surgery In 10 patients with no evidence of skeletal abnormality (HP <23 mg/24 h/m² AFOS <260 U/l and normal bone X rays) no values less than 8 0 mg/100 ml have been observed and Ca supplement has been unnecessary

In bone involving diseases there is usually a parallelism between changes in AFOS and HP values and the underlying causes of the few dissociations between these determinations have been discussed (6 12) In the present patient the different changes in these two laboratory parameters seem obvious after removal of the adenoma the elevation of AFOS reflects increased osteoblastic bone formation which probably also explains the slightly elevated HP values after the operation The rapid postoperative decrease in HP excretion is most probably due to the fact that increased HP excretion in hyperparathyroidism and in several other metabolic bone diseases derives from an increased rate of bone resorption and to a lesser extent from an increased rate of bone formation

High urinary free HP levels (in this case over 200×normal) were due to the decreased renal clearance of free HP The preoperative clearance values varied within 6 6–11 3 ml/min/1 73 m² (normal adult 0 1–0 6) and fell somewhat during 2 days postoperatively (2 6 and 2 3 ml/min/1 73 m²) but rose again to 3 7–11 8 ml/min/1 73 m² during an observation period of 4 months Since postoperative decreases in the free HP concentrations (5 2 and 4 7 μg/ml during the 2 first postoperative days and 3 0–6 9 μg/ml during 0–4 months after operation) and PTH (2 1–4 3 mg/ml respectively) were not followed by similar decreases in the clearance of free HP it seems that neither the relatively high serum free HP levels nor the direct effect of PTH on the clearance of free HP was the main cause of the high clearance rate of free HP The increased clearance rates are more probably due to the impairment of renal function caused by prolonged hyperparathyroidism This assumption is supported by the finding that the previous cases with an increased ratio of free/total HP in this hospital have shown at least some impairment in the renal function tests as well (3 6) The present patient had a low creatinine clearance (30–46 ml/min/1 73 m²) In fact in some cases such as this one the very high urinary free HP output was of diagnostic value since this phenomenon has been observed in addition to hyperparathyroidism only in hydroxyprolinaemia a very rare type of aminoaciduria (10)

Considering the immunochemical heterogeneity of PTH the different disappearance rates of different hormonal fractions and consequently the difficulties in assaying the biological activity of PTH (1 2 11) the similar changes in immunoreactive PTH and total HP excretion are in harmony with the experimental findings that the rate of bone catabolism serves as a final moderator or messenger in the hormonal regulation of Ca homeostasis (4 5 7) and call for further studies on the possible metabolic interactions between the suggested second messengers (cAMP Ca^{++} and biologically active vitamin D) and bone collagenous matrix metabolism

REFERENCES

1 Almqvist S Hjern B & Wasthed B The diagnostic value of a radioimmunoassay for parathyroid hormone in human serum Acta Endocrinol (Kbh) 78 493 1975
2 Habener J F Mayer G P Dee P C & Potts J T Jr Metabolism of amino- and carboxyl sequence immunoreactive parathyroid hormone in the bovine evidence for peripheral cleavage of hormone Metabolism 25 385 1976
3 Kalima T Kivirikko K I Laitinen O & Uitto J Urinary free and total hydroxyproline and the effect of removal of parathyroid adenoma Clin Chim Acta 19 443 1968
4 Laitinen O Parathyroid induced changes in collagen and calcium metabolism in vivo Endocrinology 80 815 1967
5 — The metabolism of collagen and its hormonal control in the rat With special emphasis on the interactions of collagen and calcium in the bones Acta Endocrinol (Kbh) (Suppl) 120 1967
6 — Clinical applications of urinary hydroxyproline determination Acta Med Scand (Suppl) 577 1974
7 — Relation to osteoporosis of age and hormone induced changes in the metabolism of collagen and bone Isr J Med Sci 12 620 1976
8 — Unpublished results

9 Parfitt A M The actions of parathyroid hormone on bone Relation to bone remodeling and turnover calcium homeostasis and metabolic bone diseases Metabolism 25 909 1976
10 Pelkonen R & Kivirikko K I Hydroxyprolinemia *An apparently harmless familial metabolic disorder* N Engl J Med 283 451 1970
11 Silverman R & Yalow R S Heterogeneity of parathyroid hormone clinical and physiological implications *J Clin Invest 52 1958 1973*
12 Smith R Dissociation between changes in urinary total hydroxyproline and plasma alkaline phosphatase *after removal of parathyroid tumor Clin Chim Acta* 23 421 1969

Acta Med Scand 202 43–45 1977

Urinary Cyclic AMP
Relation to Calcium Balance and Comparison of Assay Methods

Bo Israelsson Folke Lindgarde and Jorgen Malmquist

From the Department of Medicine University of Lund Malmo General Hospital Malmo Sweden

ABSTRACT Urinary adenosine 3 ,5 monophosphate (cyclic AMP, cAMP) has been determined in 84 males Two protein binding assays were used the method of Gilman and the Amersham assay kit The results were in close agreement The excretion of cAMP was not correlated to urinary calcium or to estimated calcium intake

Parathyroid hormone influences renal handling of calcium and phosphate by modulating the synthesis of cyclic AMP (cAMP) in tubular cells (1) Determination of urinary cAMP (U cAMP) is therefore of value as an index of parathyroid activity e g in the differential diagnosis of hypercalciuria After a calcium load urinary calcium (U Ca) will increase while except in hyperparathyroidism U cAMP decreases (9 10)

The present study aimed at investigating possible relationship between estimated dietary calcium intake U Ca and U cAMP In addition the samples were used for a comparison of the widely used cAMP assay of Gilman (4) with a recently introduced assay kit based on the procedure of Tovey et al (15)

SUBJECTS AND PROCEDURES

In the City of Malmo males aged 49 were invited to a multiphasic screening procedure at the Division of Preventive Medicine Department of Medicine For the present study 84 individuals were taken at random from those who had a normal outcome of the screening procedure which included serum phosphate and serum calcium determinations Seven persons (8 3%) in the study group had a history of urinary tract lithiasis Five of these and an additional four had a family history of renal stones

---

Requests for reprints to B Israelsson Department of Medicine Malmo General Hospital S 21401 Malmö Sweden

Blood and urine samples were obtained during Oct–Dec 1975 The subjects were instructed to collect 24 hour urine volumes in plastic vessels containing 25 ml of 5 mol/l hydrochloric acid Habitual calcium intake was estimated from answers to a questionnaire concerning average daily intake of milk and dairy products The subjects were also questioned regarding regular drug use

ASSAY METHODS

Urinary calcium was determined by atomic absorption spectrometry Urinary creatinine was measured by the Technicon AutoAnalyzer adaptation of the Jaffe reaction

Urinary cAMP was determined according to Gilman (4) as well as with the Cyclic AMP Assay Kit (TRK 432) of the Radiochemical Centre Amersham UK (15)

Gilman method Protein kinase and protein kinase inhibitor as well as cAMP standard were from Sigma Chemical Co Tritium labeled cAMP was obtained from New England Nuclear Millipore type HAMK 02412 filters and a Millipore 3025 Sampling Manifold and vacuum pump were used Urine samples were diluted 100 fold in assay buffer Assay conditions were as specified by Gilman The filters were placed in scintillation vials containing 1 ml methyl cellosolve for dissolution Ten ml toluene methyl cellosolve (3 1) containing 0 5 g PPO and 0 03 g POPOP/100 ml were added and radioactivity was measured in a Packard Tri-Carb counter

Amersham method Urine samples were diluted 50-fold in assay buffer The assay kit was used exactly according to the manufacturer s directions After the charcoal separation step 200 μl of supernatant were transferred to scintillation vials containing 10 ml of the above fluor solution All assay components (except scintillation fluid) were contained in the assay kit

Statistical calculations were made by Student s *t* test and linear regression analysis

RESULTS

Determinations of U cAMP by the two methods gave similar results the Gilman values tending to be somewhat higher (Table I) A high degree of correlation was found between the two series of data (Table I Fig 1) The magnitude of cAMP excretion

9 Parfitt A M The actions of parathyroid hormone on bone Relation to bone remodeling and turnover calcium homeostasis and metabolic bone diseases Metabolism 25 909 1976
10 Pelkonen R & Kivirikko K I Hydroxyprolinemia An apparently harmless familial metabolic disorder N Engl J Med 283 451 1970
11 Silverman R & Yalow R S Heterogeneity of parathyroid hormone clinical and physiological implications J Clin Invest 52 1958 1973
12 Smith R Dissociation between changes in urinary total hydroxyproline and plasma alkaline phosphatase after removal of parathyroid tumor Clin Chim Acta 23 421 1969

phosphate compounds in urine and the absence of significant amount of protein in most samples are advantageous factors

Urinary cAMP in relation to calcium data

The study was conducted during a brief period which is an advantage considering the seasonal variations noted in urinary calcium excretion (12 16) Most of the dietary calcium originates from milk and dairy products Accordingly information on the consumption of these nutriments gives a rough estimate of daily calcium intake We found no correlations between estimated calcium intake urinary calcium and urinary cAMP In addition to the limited precision of the estimation of calcium intake interindividual variations in intestinal calcium absorption are of obvious importance Several previous studies have shown a similar lack of correlation between calcium intake and urinary calcium (8 14 17) With regard to U cAMP the data may be taken to suggest that ordinary variations in calcium intake do not sufficiently influence parathyroid hormone secretion to cause corresponding variations in U-cAMP From a practical standpoint this means that variations in dietary habits generally need not be taken into account in the interpretation of U-cAMP data In contrast acute calcium loading is able to influence U-cAMP through suppression of parathyroid hormone secretion (9 10) This is because acute loading does not allow time for an adaptive decrease in intestinal calcium absorption

Hypercalciuria is commonly found in individuals with renal stone disease (2 8 13) Although the stone formers in the present study tended to have higher calcium intake and urinary calcium than the others they did not deviate significantly from the remaining individuals regarding urinary cAMP The latter finding is explicable on the basis of the existence of various forms of hypercalciuria with differing relations to urinary cAMP (9 10)

REFERENCES

1 Aurbach G D Marcus R Heersche J & Marx S Hormones and other factors regulating calcium metabolism Ann NY Acad Sci 185 386 1971

2 Coe F L & Kavalach A G Hypercalciuria and hyperuricosuria in patients with calcium nephrolithiasis N Engl J Med 291 1344 1974

3 Gennari C Galli M & Montagnani M Urinary cyclic adenosine monophosphate in young adults and elderly subjects J Clin Path 29 69 1976

4 Gilman A G A protein binding assay for adenosine 3 5 -cyclic monophosphate Proc Natl Acad Sci USA 67 305 1970

5 Guttler R B Shaw J W Otis C L & Nicoloff J T Epinephrine induced alterations in urinary cyclic AMP in hyper and hypothyroidism J Clin Endocrinol Metab 41 707 1975

6 Hamet P Lowder S C Hardman J G & Liddle G W Effects of hypoglycemia on extracellular levels of cyclic AMP in man Metabolism 24 1139 1975

7 Nistrup Madsen S Badawi I & Skovsted L A simple competitive protein binding assay for adenosine 3 ,5 monophosphate in plasma and urine Acta Endocrinol 81 208 1975

8 Nordin B E C Peacock M & Wilkinson R Hypercalciuria and calcium stone disease Clin Endocrinol Metabol 1 169 1972

9 Pak C Y C Kaplan R Bone H Townsend J & Waters O A simple test for the diagnosis of absorptive resorptive and renal hypercalciurias N Engl J Med 292 497 1975

10 Pak C Y C Ohata M Lawrence E C & Snyder W The hypercalciurias Causes parathyroid functions and diagnostic criteria J Clin Invest 54 387 1974

11 Raij K & Härkonen M Determination of cyclic adenosine 3 ,5 monophosphate in urine Scand J Clin Lab Invest 36 161 1976

12 Robertson W G Gallagher J C Marshall D H Peacock M & Nordin B E C Seasonal variations in urinary excretion of calcium Br Med J 4 436 1974

13 Robertson W G & Morgan D B The distribution of urinary calcium excretions in normal persons and stone formers Clin Chim Acta 37 503 1972

14 Spencer H Lewin I Fowler J & Samachson J Influence of dietary calcium intake on Ca^{47} absorption in man Am J Med 46 197 1969

15 Tovey K C Oldham K G & Whelan J A M A simple direct assay for cyclic AMP in plasma and other biological samples using an improved competitive protein binding technique Clin Chim Acta 56 221 1974

16 Transbøl I Jørgensen F S Lund B & Sørensen O H Importance of season and clearance correction in the definition of hypercalciuria Acta Med Scand 199 127 1976

17 Wills M R Intestinal absorption of calcium Lancet 1 820 1973

Acta Med Scand 202 47–50 1977

Bone Composition and Vitamin D after Pólya Gastrectomy

L Tougaard H Rickers P Rodbro E Hess Thaysen
Merete Sanvig Christensen B Lund and O Helmer Sörensen

From the Departments of Clinical Physiology Internal Medicine (Division of Gastroenterology) and Clinical Chemistry Ålborg Hospital Ålborg the Institute of Pharmacology University of Århus Århus and Department of Internal Medicine E Frederiksberg Hospital Copenhagen Denmark

ABSTRACT With the aim of evaluating bone phosphorus/hydroxyproline ratio (P/Hypro) as an index of osteomalacia, this bone index and the bone mineral content (BMC) have been investigated together with other indices of calcium metabolism in 27 gastrectomized patients None of the patients had clinically manifest bone symptoms The mean values of bone P/Hypro BMC, plasma calcium and plasma magnesium were subnormal the mean values of serum parathyroid hormone (iPTH) and plasma alkaline phosphatase were elevated Mean serum 25 hydroxycholecalciferol (25-OH D) did not differ from normal A significant positive correlation was found between bone P/Hypro and serum 25 OH D but no significant correlation between bone P/Hypro and BMC Serum 25 OH D and bone P/Hypro were significantly lower and serum iPTH was significantly higher in a subgroup of 12 patients with no regular supplementary intake of vitamin D In conclusion, the gastrectomized patients had blood biochemical evidence of a mild vitamin D insufficiency and the low bone P/Hypro values can be explained by mild osteomalacic changes in bone

Partial gastrectomy may expose the patient to intestinal malabsorption with possible vitamin D deficiency (13 15) and calcium deficiency (7 11 12 16) This may result in bone abnormalities (1 2 3 7 8 9 10 15 19) characterized either by osteomalacia when vitamin D deficiency is the main problem or by osteoporosis when other factors are mainly reponsible Both osteoporosis and osteomalacia are characterized by bone rarefaction and decreased bone mineral content (BMC) In osteoporosis bone histology is qualitatively normal but the BMC reduced Osteomalacia is characterized by an excessive amount of unmineralized bone matrix (osteoid) besides a reduced BMC The differential diagnosis between osteomalacia and osteoporosis is important (18) but not easily obtained It can be reached by quantitative histological examination of large bone biopsies handled and evaluated by experts Osteomalacia might also be diagnosed by chemical measurement of the phosphorus to hydroxyproline ratio (P/Hypro) in minute bone biopsies (21) This ratio estimates the proportion of inorganic substance (mainly different calcium phosphates) to organic matrix in bone (mainly collagen) and the bone P/Hypro is accordingly an estimate of the degree of mineralization in bone In osteoporosis normal values of bone P/Hypro are found (22) whereas low values of this ratio are expected in osteomalacia

To evaluate the bone P/Hypro in vitamin D deficiency this index and the BMC were measured in patients with Polya gastrectomy together with serum 25 hydroxycholecalciferol (25 OH D) and other chemical quantities in blood concerning bone metabolism

PATIENTS AND METHODS

Patients

A group of 34 patients with Polya (Billroth II) gastrectomy were approached and 27 (23 males and 4 females aged 40–84 years mean 62) agreed to participate in the study during Oct –Nov 1975 The patients had undergone the gastrectomy for duodenal ulcer in the period 1958–68 at the Department of Surgery Ålborg Municipal Hospital the mean interval from operation to the present study being 13 years Twenty three patients were randomly selected from the survivors of a consecutive series of gastrectomized patients previously reevaluated at the Department of Medicine and four were included after applications to general practitioners The latter patients did not

Table I *Plasma and bone indices in 27 patients with Polya gastrectomy compared with reference values (mean±S D)*

	Patients	Controls	Significance of difference
Plasma			
25 hydroxycholecalciferol (ng/ml)	25 6±13 1	28 0±9 6	n s
Parathyroid hormone (log pg/ml)	1 94±0 25	1 75±0 14	$p<0\ 01$
Calcium (mmol/l)	2 36±0 12	2 50±0 15	$p<0\ 001$
Magnesium (mmol/l)	0 86±0 08	0 95±0 08	$p<0\ 001$
Phosphorus (mmol/l)	1 18±0 20	1 18±0 19	n s
Alkaline phosphatase (log U/l)	2 33±0 16	2 08±0 19	$p<0\ 001$
Protein (g/l)	73 8±4 4	73 0±5 0	n s
Bone			
Mineral content (% of normal)	87 8±18 8	100 0±16 5	$p<0\ 01$
Phosphorus/hydroxyproline (% of normal)	95 2±7 2	100 0±7 0	$p<0\ 01$

differ from the former concerning age time of operation or symptoms

Though the presence of bone disorders was disregarded in the selection none of the patients had clinically manifest bone disease All patients were asked about supplementary vitamin D intake None of them were receiving therapeutic doses of vitamin D preparations Twelve patients had regularly taken supplementary vitamin D in the form of a daily multivitamin tablet containing 600 IU cholecalciferol (vitamin D group mean age 60 years) Fifteen patients (mean age 63 years) did not take drugs containing vitamin D regularly

Investigations

The patients were not fasting at the time of blood sampling Plasma concentrations of calcium protein inorganic phosphorus and alkaline phosphatase were measured on a Technicon SMA® 12/60 autoanalyser system Plasma magnesium was determined by atomic absorption Serum 25 OH D was measured by competitive protein binding assay (14) Serum parathyroid hormone (iPTH) was measured by radioimmunoassay on extracts of serum using AS 211/32 and bovine PTH (4) BMC was measured by photon absorptiometry in the forearm (6) The degree of bone mineralization was measured as the bone P/Hypro in bone biopsies obtained in local anaesthesia (21) The intraindividual variation of bone P/Hypro in the patients was determined from duplicate measurements on the biopsies coefficient of variation 5 9% (in normal subjects 5 0% (21)) The means of the duplicate determinations were used in the study As the normal bone P/Hypro depends on age (22) and the normal BMC depends on sex and age (5) the two bone indices were expressed as per cent of the corresponding normal mean

Statistical analysis

The reference values of serum 25 OH D were measured in normals at the same time of the year as the investigation was performed The other normal values were either the reference values at our Department of Clinical Chemistry or have been published elsewhere (4 5 22) Values of serum iPTH and plasma alkaline phosphatase were logarithmically transformed Student s *t* test or the Mann Whitney *U* test were used to compare mean values

RESULTS

Table I shows the means and S D of measurements in patients and controls Plasma calcium and plasma magnesium were decreased whereas serum iPTH and plasma alkaline phosphate were increased Serum 25 OH D plasma protein and plasma phosphorus were normal Both bone P/Hypro and BMC were decreased

Bone P/Hypro decreased significantly with decreasing values of serum 25 OH D (Fig 1) The coefficient of correlation was 0 51 ($p<0\ 01$) for all patients and after exclusion of one patient with extremely low values of bone P/Hypro and serum 25 OH D 0 42 ($p<0\ 05$) Bone P/Hypro did not correlate to serum iPTH ($r=-0\ 08$ n s) plasma calcium ($r=0\ 16$ n s) or BMC ($r=0\ 27$ n s) No significant correlation was found between BMC and serum 25 OH D ($r=0\ 03$ n s) BMC and serum iPTH ($r=0\ 09$ n s) or BMC and plasma calcium ($r=0\ 05$ n s) Neither serum 25 OH D nor serum iPTH correlated significantly to plasma calcium ($r=0\ 36$ n s and $r=-0\ 25$ n s respectively) Neither BMC nor bone P/Hypro correlated to the time since gastrectomy ($r=-0\ 21$ n s and $r=-0\ 20$ n s respectively)

In patients with no regular vitamin D supplements mean serum 25 OH D and mean bone P/Hypro were significantly lower and mean serum iPTH significantly higher than in the vitamin D

Table II *Plasma and bone indices in patients with Polya gastrectomy grouped according to supplementary vitamin D intake (mean±S D)*

	Regular vitamin D supplement (n=12)	No regular vitamin D supplement (n=15)	Significance of difference
Plasma			
25 hydroxycholecalciferol (ng/ml)	32 1±15 0	20 3±8 6	p<0 05
Parathyroid hormone (log pg/ml)	1 81±0 28	2 03±0 21	p<0 05
Calcium (mmol/l)	2 36±0 13	2 36±0 11	n s
Magnesium (mmol/l)	0 85±0 05	0 87±0 09	n s
Phosphorus (mmol/l)	1 14±0 16	1 21±0 24	n s
Alkaline phosphatase (log U/l)	2 28±0 10	2 38±0 19	n s
Protein (g/l)	73 2±3 5	74 4±4 2	n s
Bone			
Mineral content (% of normal)	83 9±15 4	90 9±21 1	n s
Phosphorus/hydroxyproline (% of normal)	98 9±6 2	92 4±6 9	p<0 02

group (Table II) No significant differences between the two groups were found in the mean plasma values of calcium protein phosphorus magnesium alkaline phosphatase or the mean BMC values

DISCUSSION

The very low correlation coefficient of serum 25 OH D and BMC is probably due to the relatively high interindividual variation of BMC (5) but may also indicate that other causes than vitamin D insufficiency can induce low BMC in gastrectomized patients The lack of correlation between the two bone indices may also indicate the independence of the two methods

Plasma magnesium was low in most patients Probably this ion (17) together with a deficit of calcium plays a significant role for some of the bone changes in patients with malabsorption or maldigestion The present data however do not allow conclusions regarding this problem

No regard was paid to the presence of bone disease in the selection of patients for the study In fact bone disease was not a serious disability for any of the patients It is in accordance with other studies of similarly selected patients (2 7 20) that the changes demonstrated in bone metabolism were mostly of only moderate severity

The low mean value of BMC however demonstrates that the patients as a group had disturbed bone metabolism Though serum 25 OH D values were largely normal other blood indices indicated a slight degree of vitamin D insufficiency (3 13 15) Therefore the low mean value of bone P/Hypro could be due to subclinical vitamin D insufficiency The significantly lower value of bone P/Hypro in the subgroup with no supplementary vitamin D intake than in the vitamin D group supports this assumption This suggests that bone P/Hypro is highly sensitive to decreased vitamin D supply from the intestine This probably explains why bone P/Hypro and serum 25 OH D correlate significantly even when the values of each index were within normal limits Only one patient had a significantly

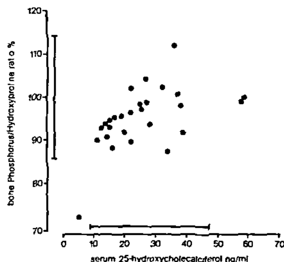


Fig 1 Serum 25-hydroxycholecalciferol and bone phosphorus/hydroxyproline ratio in gastrectomized patients Vertical and horizontal bars indicate normal range (mean ±S D) The correlation is significant

decreased value of serum 25 OH D (Fig 1) and this patient also had a subnormal value of bone P/Hypro

Very low values of bone P/Hypro will probably be *found in patients with clinically man fest symp* toms of osteomalacia

REFERENCES

1 Alhava E M Aukee S Karjala nen P Kettunen K & Juut M The influence of calc um and *calc um+v tam n D_2 treatment on bone m neral after* partial gastrectomy Scand J Gastroenterol 10 689 1975

2 Aukee S Alhava E M & Karjala nen P Bone m neral after part al gastrectomy II Scand J Gastroenterol 10 165 1975

3 Chalmers J Conacher W D H Gardner D L & Scott P J Osteomalac a—a common d sease in elderly women J Bone Jo nt Surg 49 B 403 1967

4 Christensen M S A sensit ve rad oimmunoassay of parathyro d hormone in human serum us ng a spec f c *extraction procedure Scand J Clin Lab Invest* 36 313 1976

5 Christ ansen C & Rodbro P Bone m neral content and est mated total body calcium in normal adults Scand J Clin Lab Invest 35 433 1975

6 Christ ansen C Rodbro P & Jensen H Bone m n eral content in the forearm measured by photon absorpt ometry Princ ples and rel ab l ty Scand J Clin Lab Invest 35 323 1975

7 Deller D J Edwards R G & Add son M Calcium metabol sm and the bones after part al gas trectomy Aust Ann Med 12 295 1963

Eddy R L *Metabol c bone d sease after gastrec* tomy Am J Med 50 442 1971

9 Garner A & Ball J Quant tative observat ons on m neral sed and unm neral sed bone n chron c renal azotaem a and intest nal malabsorption syndrome J Path Bact 91 545 1966

10 Garrick R Ireland A W & Posen S Bone ab normal t es after gastric surgery Ann Intern Med 75 221 1971

11 Gregory D H & van Uelft R Calc um absorpt on follow ng gastric resect on Am J Gastroen erol *57 34 1972*

12 Harvald B Krogsgaard A R & Lous P Calc um def c ency follow ng partial gastrectomy Acta Med Scand 172 497 1962

13 Jaworski Z F G Pathophys ology d agno s and treatment of osteomalacia Orthop Cl n North Am 3 623 1972

14 Lund B Sorensen O H & Christensen A B 25 hydroxycholecalciferol and fractures of the prox mal femur Lancet 2 300 1975

15 Morgan D B Hunt G & Peterson C R The osteomalac a syndrome after stomach operat ons Q J Med 155 395 1970

16 Morgan D B Pulvertaft C N & Fourman P Effects of age on the loss of bone after gastric surgery Lancet 2 772 1966

17 Muldowney F P McKenna T J Kyle L H Freaney R & Swan M Parathormone l ke effect of *magnes um replenishment n steatorrhea* N Engl J Med 281 61 1970

18 Newton John H F & Morgan D B The lo s of bone with age osteoporosis and fractu es Cl n Orthop 71 229 1970

19 Nilsson B E & Westl n N E Femur den y alcohol sm and after gastrectomy Calc f T s ue R 10 167 1972

20 Pryor J O O Shea M J Brooks P L & Data C K The long term metabol c consequences of partı gastrectomy Am J Med 51 5 1971

21 Tougaard L The degree of m neral zat on n bon *t ssue The* phosphorus/hydroxyprol ne rat o dete m ned on small amounts of bone t ssue Scand J C Lab Invest 32 351 1973

22 — The degree of m neral zat on in normal bone u est mated as the phosphorus to hyd oxyprol ne ra r Scand J Cl n Lab Invest 35 285 1975

Acta Med Scand 202 51–54 1977

The Urinary Sediment in Endemic Benign Nephropathy

A Phase Contrast Microscopy Study

Anders Wahlin Bengt Lindqvist and Kurt Nyström

From the Department of Medicine University Hospital Umea Sweden

ABSTRACT Urinary sediments from 30 patients with endemic benign (epidemic) nephropathy (EBN) have been examined with phase contrast microscopy Casts often with an irregular outline, were found in 80% of the patients most commonly in those showing the most advanced impairment of kidney function as judged by the maximum concentration of serum creatinine registered during the acute phase of the disease Conspicuously thin and long thread like objects of a kind not previously reported in the literature on the urinary sediment were seen in 40% of the patients they possibly represented casts formed in the loop of Henle Leukocyturia (>5 leukocytes per visual field) was present in 20% of the cases A number of cells sufficient for differential count was found in nearly all urine specimens examined 15% granulocytes 35% mononuclear leukocytes 50% renal epithelial cells (REP) (mean values), a proportion of REP greater than that found at phase contrast microscopy of the urinary sediment in kidney diseases other than EBN Microscopic hematuria of a few days duration was found in 10% of the cases a figure considerably smaller than previously reported The findings seem to be almost pathognomonic for EBN and most of them were readily explained by the histopathologic changes found in percutaneous needle biopsy specimens of the kidney during the acute phase of the disease

Endemic benign (epidemic) nephropathy (EBN) is a disease of undetermined etiology occurring in Northern Scandinavia Its cardinal symptoms and signs are fever with a sudden onset pain in the abdomen and in the back proteinuria and a raised serum creatinine concentration (5 12 15)

Although nearly 1 000 cases of the disease have been reported in Scandinavia since the first description in 1934 (15 20) controversy still seems to prevail concerning the sedimentary findings during the acute phase They have been described as scanty (2 3 4 5 6 8 9 14) or as consisting predominantly of casts (7 17 18) or erythrocytes (12 16 17 19) Leukocytes have been found in moderate amounts and only infrequently (5 6 7 8 14 19) Mouland (13) reports the presence of large cells with cytoplasmic inclusions in the urinary sediment of EBN cases In hemorrhagic fever with renal syndrome a disease suggested to be related to EBN (12) polynuclear giant cells and mononuclear cells probably representing renal epithelial cells have been found in the urinary sediment

The aim of the present study was to examine the urinary sediment in EBN with respect to the presence of cells and casts and to relate the results to what is known about the renal histopathological changes in the acute phase of the disease

PATIENTS AND METHODS

Nineteen males aged 33 (range 16–50) and 11 females aged 38 (range 21–58) years were investigated as in patients in our department

The patients were requested to empty the bladder and then drink two glasses of water One to two hours later a 10 ml sample of voided urine was collected and immediately centrifuged at 2 500 r p m for 10 min The supernatant was carefully poured off and the sediment resuspended by finger flipping Using a drinking straw a minute amount of the sediment (less than a drop) was placed on a slide and coverglass applied thereby creating a very thin liquid film necessary for good optical resolution Examination was immediately performed in a phase contrast microscope at a magnification of ×400 Occurrence type and shape of casts was recorded and the number of erythrocytes leukocytes and renal epithelial cells (REP) was counted in 10 visual fields Oil immersion and a magnification of ×1 000 was used for identification

of REP and type of leukocytes 50-100 cells were examined The latter cell category was divided into granulocytes and mononuclear leukocytes the criteria used for cell identification have been described earlier (10) Squamous epithelial cells and urothelial cells were not included in the differential count

The initial examination was performed within 1–2 weeks after the onset of the disease in 13 cases up to five serial examinations were carried out In 10 cases reexamination was performed within 2–4 months A total of 54 sediments were examined three of which gave no information about the urinary cell distribution because of too few cells at one (initial) examination and because salts and mucous threads obscured the cells at two (late) examinations

RESULTS

Casts were found in great or moderate amounts in 18 patients (60%) whose mean maximum serum creatinine concentration was 7 8 mg/100 ml In 12 patients (40%) casts were not observed (six cases) or their number was regarded as insignificant (six cases maximum two casts observed in each specimen) The mean maximum serum creatinine concentration in this group was 4 5 mg/100 ml The difference in maximum serum creatinine concentration between the two groups was significant when tested statistically using Wilcoxon s rank sum test for independent samples ($p<0\ 05$)

Hyaline casts were found in 16 patients granular 13 cellular containing REP in 12 and waxy casts in two

In five patients very long and broad casts were found extending over more than six visual fields at a magnification of ×400 (Figs 1 2 8) In 12 patients granular and hyaline casts were seen that were either wavy (Fig 3) or whose contour was knobbed (Fig 4) or indented (Fig 5) Curled casts (Fig 10) were also seen Another prominent observation was conspicuously thin and sometimes very long objects (Figs 7 9) some of which displayed a distinct longitudinal structure that sometimes appeared to be spirally wound (Figs 1 6) and whose exact nature remains obscure They probably did not represent so-called mucous threads They were observed in 12 patients We have found no description of similar objects in the urine nor were any structures resembling these observed by us in urinary sediments from patients with kidney diseases other than EBN (10)

Composite casts were also found consisting of broad hyaline casts containing slender ones of a different type (Figs 1 8 9) a finding consistent with casts being formed at different levels of the nephron

Leukocytes and renal epithelial cells An increased number of leukocytes (>5 per visual field was found in six patients (20%) A sufficient number of cells for differential count was found in nearly all urine specimens examined a mean of 15% granulocytes (range 4–72) 35% mononuclear leukocytes (range 2–60) and 50% REP (range 9–80)

A few large cells of obscure but probably *urothelial* origin containing large cytoplasmic vacuoles were sometimes found (Fig 11) Also multinucleated giant cells were found in some cases

Erythrocytes In three patients (10%) microscopic hematuria (>3 erythrocytes per visual field) was found persisting for a few days In one patient it was prominent at the initial examination but absent two days later

Microorganisms Bacteria or fungi were not observed

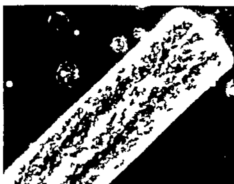
DISCUSSION

Several factors characterizing the acute phase of EBN probably favour the formation of casts Proteinuria is almost always present and sometimes heavy In advanced cases the intrarenal blood flow is impeded (11) the kidneys are diffusely swollen and the patients are oliguric for a few days The main histopathological kidney lesion consists of an interstitial inflammation and signs of degenerative changes in the tubular epithelium (12) The wavy contour and the indentations seen in some of the casts might be caused by impression of swollen tubular cells The thread like objects may be casts formed in the loop of Henle The degree of kidney damage seems to be reflected in the number of casts shed with the urine

In an earlier cytological investigation (10) patients with EBN had a greater proportion of REP in the urine than patients with other kidney diseases, probably reflecting the degenerative changes in the

Figs 1 2 and 8 depict parts of broad very long casts extending over several visual fields Thread like objects are depicted in Figs 1 6 7 and 9 Composite casts are shown in Figs 1 8 and 9

Photographs were taken through a phase contrast microscope at a magnification of ×254 (Figs 1 10) and ×635 (Fig 11)


1


2


3


4


5


6


7


8


9


10


11

Fg 1 Granular cast conta n ng a slender thread l ke object that appears to be sp rally wound
Fg 2 Hyal ne cast w th nclus ons consist ng of debris
Fg 3 Granular cast with a wavy contour
Fg 4 Granular cast w th a knobbed contour
Fg 5 Hyal ne cast with ndentat ons
Fg 6 Thread l ke f lamentous structure that appears to be sp rally wound

F g 7 Thread l ke granular cast
F g 8 Broad f nely granulated cast surround ng a coarsely granulated one
F g 9 Hyal ne cast contain ng a f lamentous thread l ke structure
F g 10 Hyal ne curled cast contain ng debr s
F g 11 Uroepithel al (?) cell with cytoplasm c vacuoles at the periphery

tubular epithelium observed at histopathological examination of kidney specimens taken during the acute phase of the disease

Leukocyturia when present was only moderate in accordance with reports by earlier investigators (13 15 18 20)

In previous studies of the urinary sediment in EBN (6 17) hematuria irrespective of degree was always of very short duration as was the case in our patients Its intensity however has been reported to range from only solitary erythrocytes (4 7 8) or in the majority of cases a moderate increase in their number (5 15 17) to macroscopic hematuria in a few patients (12 17) The incidence of hematuria reported in EBN also varies considerably the highest figure being 75% (12) In our patients hematuria was rare and frank microscopic hematuria was seen only once

In the majority of instances hematuria in EBN is probably not caused by glomerular damage as no significant hemorrhagic lesions were found in the glomeruli at light and transmission electron microscopy of needle biopsy specimens from the kidney taken during the acute phase (1 12) The inflammatory reaction of the medullary interstitium characteristic of EBN is sometimes hemorrhagic (9 12) and in one case hemorrhages have been found to extend into the lumina of the tubuli (17) Therefore hematuria in EBN might be of tubular origin which [illegible] explain its short duration and wide variations [illegible] degree reported in the literature

In our experience careful examination of the urinary sediment by phase contrast microscopy including differential cell count reveals a picture which probably is pathognomonic for EBN

REFERENCES

1 Collan Y & Lahdevirta J Glomerular changes in nephropathia epidemica Scand J Clin Lab Invest (Suppl) 113 25 1970

2 Coster C & Lublin H So-called nephropathia epidemica Acta Med Scand (Suppl) 246 46 1950

3 Dolk H Nephropathia epidemica i Mellansverige (Varmland) Nord Med 71 443 1964

4 Etholm B Nephropathia epidemica Tidsskr Nor Laegeforen 80 386 1960

5 Fjellstrom K E Nephropathia epidemica in Uppland Acta Med Scand 167 111 1960

6 Hansen E Atypisk akut nefritis Kliniske iakttagelser i en raekke tilfaelde Ugeskr Laeg 120 908 1958

7 Hortling H En epidemi av faltfeber (?) i finska Lappland Nord Med 30 1001 1946

8 Knutrud O Nephropathia epidemica Tidsskr Nor Laegeforen 69 259 1949

9 Kuhlback B Fortelius P & Tallgren L G Renal histopathology in a case of nephropathia epidemica Myhrman A study of successive biopsies Acta Pathol Microbiol Scand 60 323 1964

10 Lindqvist B & Wahlin A Differential count of urinary leucocytes and renal epithelial cells by phase contrast microscopy Acta Med Scand 198 505 1975

11 Lingårdh G Lundstrom B & Nystrom K Renal blood flow in acute epidemic nephritis (nephropathia epidemica of Scandinavia) Scand J Urol Nephrol 9 57 1975

12 Lahdevirta J Nephropathia epidemica in Finland A clinical histological and epidemiological study Ann Clin Res (Suppl) 8 1971

13 Mouland C Nephropathia epidemica 12 kasus fra Grane og Hattfjelldal i Nordland Fylke august 1973–januar 1974 Tidsskr Nor Laegeforen 95 476 1975

14 Muri J W Nephropathia epidemica Report of a case J Oslo City Hosp 3 192 1953

15 Myhrman G En njursjukdom med egenartad symptombild Nord Med T 7 793 1934

16 — Nephropathia epidemica a new infectious disease in Northern Scandinavia Acta Med Scand 140 5? 1951

17 Ornstein K & Soderhjelm L Nephropathia epidemica Med Welt 17 898 1965

18 Stuhlfaut K Bericht uber ein neues schlammfieberahnliches Krankheitsbild bei deutschen Truppen in Lappland Dtsch Med Wochenschr 69 439 474 605 1943

19 Tungland B Nephropathia epidemica Nord Med 51 635 1954

20 Zetterholm S G Akuta nefriter simulerande akuta bukfall Lakartidningen 31 425 1934

Acta Med Scand 202 55–60 1977

Blood Pressure and Renin during Treatment with Pindolol

F Fyhrquist K Kurppa M Huuskonen and A Koistinen

From the Minerva Institute for Medical Research Unit of Clinical Physiology and the IV Department of Medicine Helsinki University Central Hospital Helsinki Finland

ABSTRACT Beta receptor blocking drugs are known to decrease BP and plasma renin activity (PRA) in hypertensive patients We treated 31 hypertensive patients with the β receptor blocking drug pindolol for 3 months During the first month (mean daily dose 10 mg) and the second month (mean daily dose 14 2 mg) BP and PRA decreased During the third month of pindolol therapy (mean daily dose 19 0 mg) 16 patients had an unexpected rise of BP towards control levels and PRA levels rose too The remaining 15 patients maintained a good antihypertensive drug effect and suppression of PRA Pretreatment PRA was not related to BP reduction The change in diastolic BP was not significantly related to that in PRA The results indicate that low doses of pindolol 10–15 mg daily, will suffice in mild essential hypertension An increasing frequency of partial drug resistance may be a result of unnecessarily high doses of pindolol

Buhler et al (4) ascribed part of the antihypertensive effect of propranolol a drug which blocks β adrenergic receptors to its capacity to suppress plasma renin activity (PRA) Other investigators have found no correlation between the antihypertensive effect and suppression of PRA during treatment with β blocking agents (1 9 10) The intrinsic β mimetic activity of pindolol also a β-receptor blocking drug makes pindolol particularly interesting to study when investigating the possible association between antihypertensive effect and the state of the renin–angiotensin system

Some investigators have reported suppression of PRA in hypertensive patients treated with pindolol (5 10 16 18) while others found no consistent reduction of PRA (2 15) Changes in PRA during pindolol treatment were not correlated with the antihypertensive drug response (2 8 9 10 15)

We studied changes in BP and PRA during pindolol treatment in patients with essential hypertension in order to clarify possible correlations between these variables

Address for reprints F Fyhrquist M D IV Department of Medicine Helsinki University Central Hospital Unioninkatu 38 SF-00170 Helsinki 17 Finland

PATIENTS AND METHODS

Patients Thirty eight outpatients with essential hypertension were randomly selected to a four month double blind trial Thirty-one patients 14 female and 17 male mean age 42 2 years (range 23–61) completed the study Excluded were patients with signs of secondary hypertension heart insufficiency obstructive pulmonary disease AV block or endocrine disorders All had supine BPs above or equal to 150/100 mmHg on three occasions during the selection period Known duration of hypertension was 2 4 years (range 0 25–10) Informed consent was obtained from all patients entering the study which was approved by the Finnish State Medical Board

Methods The examinations included ECG chest X ray rapid sequence i v pyelography and measurements of urinary 24 hour excretion of sodium and potassium Serum concentrations of sodium potassium and creatinine were determined with a SMA 12/60 Technicon AutoAnalyzer PRA was assayed with a radioimmunoassay for angiotensin I (8)

Study design The study covered four months The patients were seen at the clinic at monthly intervals BPs were recorded with a mercury manometer (diastolic pressures at the disappearance of the Korotkoff sounds) after 5 min supine rest and after 2 min standing All visits to the clinic were made at 9 00–11 00 a m Samples for PRA determination were drawn after 2 hours of upright posture and 12 hours of fasting including avoidance of alcohol coffee and smoking The same two doctors examined the same patients on their visits to the clinic

Placebo was given twice daily during the first month Pindolol 5 mg twice daily was given during the first month of active drug therapy The drug dose was increased when the antihypertensive response was consid-

Table I *Blood pressure pulse heart volume ECG variables during the study in the whole patient group*

	Selection period	Placebo month	Pindolol treatment Month 1 (10 0 mg/day)	Month 2 (14 2 mg/day)	Month 3 (19 0 mg/day)
BP					
Supine systolic	169±2 7	165±3 2	142±2 7***	144±2 9***	154±3 7**
Supine diastolic	106±1 1	106±1 1	92±1 7***	93±1 8***	98±2 0 **
Upright systolic	165±2 6	159±2 4	143±2 5***	143±2 8***	148±3 4**
Upright diastolic	105±1 0	105±1 1	93±1 8***	95±1 8***	98±1 9***
Pulse					
Supine	74±1 1	73±1 0	65±0 9***	63±1 2***	64±1 1**
Upright	73±0 8	73±1 0	68±1 0***	65±1 2***	68±1.3**
Heart volume (cm³)		427±14			458±12*
ECG					
S_1+R_5 (mm)		27±1 5			22±2 0*
P–Q time (sec)		0 148±0 004			0 156±0 004*

Significance of changes compared with values at end of placebo period * $p<0$ 05 ** $p<0$ 005 *** $p<0$ 001

ered insufficient that is >150/95 mmHg in either the supine or the standing position Side-effects were registered using a fixed inquiry schedule

Statistical evaluation All values are given as mean ± standard error of the mean (S E M) unless otherwise stated Correlations were evaluated with linear regression analysis (12) significance of changes with Student s *t*-test.

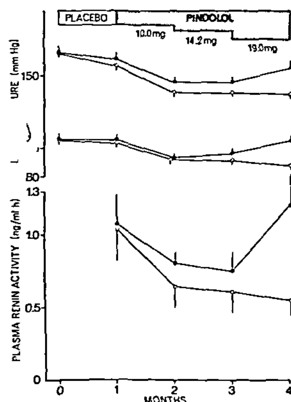


Fig 1 Systolic and diastolic BPs (supine) and PRA levels during the study O=responders ($N=15$) ●=patients with unsatisfactory response ($N=16$) vertical bars= S E M

RESULTS

Blood pressure The systolic BPs both in the supine and in the standing position decreased during the placebo month (Tables I and II)—the diastolic pressures did not Pulses did not change during placebo

Compared with the values at the end of the placebo month BP values remained decreased throughout the study (Table I) During month 3 when the mean daily dose of pindolol was increased to 19 0 mg the BPs rose significantly

Further analysis of the BP data revealed that 10 patients only were non responders (supine or upright diastolic BP ≥100 mmHg) after one month of pindolol treatment (Table II) After three months 16 patients belonged to this group despite increasing drug doses The non responders after one month of drug therapy comprised 4 females and 6 males after three months 2 females and 14 males

Cardiac variables Pulses decreased during pindolol therapy and did not increase during the third month of drug treatment (Table I) However in the group of non responders pulse rate rose during the last month of active drug therapy ($p<0$ 025) in the upright but not in the supine position The P–Q time and the heart volume as evaluated from chest X rays increased significantly (Table I) The S_1+R_5 of the ECG was reduced during pindolol treatment

Renin electrolytes and other variables PRA de-

Table II *Individual blood pressure and pulse rate during the study (upright position)*

Pat no	Sex	BP (mmHg) After placebo	BP Pindolol treatment Month 1	BP Pindolol treatment Month 2	BP Pindolol treatment Month 3	Pulse rate After placebo	Pulse rate Pindolol treatment Month 1	Pulse rate Pindolol treatment Month 2	Pulse rate Pindolol treatment Month 3
1	♂	150/100	125/85	130/90	125/85	72	62	58	60
2	♀	170/105	150/95	150/85	150/85	68	62	62	64
4[a]	♂[b]	160/100	160/100	155/100	155/105	72	68	64	68
5	♂	145/100	135/85	145/90	155/100	66	66	58	72
6	♀	140/100	150/90	155/90	165/90	68	62	62	64
7[a]	♂[c]	190/115	170/110	150/105	190/120	72	58	56	56
8	♂	160/100	150/100	165/100	170/90	66	64	64	84
9	♀	190/115	160/102	150/100	155/100	76	76	70	76
10[a]	♂	160/100	130/85	135/85	145/105	68	62	64	68
11	♀[b]	140/100	125/90	130/100	120/95	72	64	68	70
13	♂	150/110	150/110	150/110	140/95	74	74	62	60
14	♀	160/110	145/90	160/100	165/100	70	74	72	76
15[a]	♂	165/110	145/95	130/90	160/105	72	72	60	66
16	♂	145/100	130/90	145/100	135/105	74	72	72	68
18[a]	♂[b]	150/100	125/80	120/80	135/95	72	62	60	60
19	♂[c]	160/115	160/95	190/120	160/115	84	68	68	68
22	♀	175/110	130/80	120/80	135/90	76	68	60	66
23	♂	140/100	135/80	145/90	150/95	60	62	58	60
25	♀	170/110	155/100	135/80	130/90	70	68	60	60
26	♀	165/105	170/100	175/95	175/90	74	70	65	65
27	♂	170/105	140/85	135/90	165/110	76	68	60	64
28	♀	160/105	130/85	130/80	145/95	78	68	66	68
29[a]	♂	165/105	145/90	150/100	180/115	70	62	60	68
30	♂	145/100	140/90	135/95	145/100	70	66	66	74
32	♂	160/105	155/100	150/95	150/105	74	72	70	76
33	♀	140/100	130/95	130/90	120/90	72	72	66	60
34	♀	165/110	120/95	120/85	105/80	84	78	70	68
35	♂[b]	175/120	160/120	140/110	155/115	84	74	74	64
37	♀	150/105	125/85	130/105	130/95	70	66	80	65
38	♀	165/100	135/105	135/100	130/80	84	80	84	90
39	♂	150/95	140/80	140/90	140/95	68	62	66	64

[a] Non responder [b] smoker [c] heavy smoker (>50 cigarettes/day)

creased during the first month of pindolol therapy and remained suppressed at the end of the second month of therapy (Tables III and IV) At the end of the study however PRA was no longer suppressed

When studied separately the patients who responded with a satisfactory BP reduction at the end of the study ($N=15$ Table III) showed continuous suppression of their PRA value (Fig 1) The patients with an unsatisfactory BP response ($N=16$ Table III) showed a significant rise of PRA during the third month of active drug treatment (Fig 1) Pretreatment PRA levels were the same in both subgroups

The change in PRA and the change in upright diastolic BP at the end of the study were not significantly correlated ($r=0\ 324$ not significant) Pretreatment PRA was not correlated to the change in diastolic BP at the end of the study ($r=0\ 28$ not significant) No correlations between changes in diastolic BP and in PRA were detected in the sub groups of antihypertensive response either Serum potassium increased and haematocrit values decreased during pindolol therapy (Table IV) The urinary excretion of sodium and potassium serum concentrations of sodium creatinine and Hb values did not change significantly

Side effects and dropouts The drug was well tolerated Side effects were minor and decreased with time Seven patients were excluded during the study One complained of severe tachycardia during placebo another felt dizzy and experienced tachycardia 1–2 hours after taking 5 mg pindolol three patients failed to attend controls one patient reported nausea and insomnia and one was afterwards found to take a diuretic drug

Table III *Individual values of plasma renin activity and sodium excretion*
N A = not available

Pat no	PRA (ng/ml h) After placebo	Pindolol treatment Month 1	Month 2	Month 3	Urinary sodium excretion (mmol/24 h) After placebo	Pindolol treatment Month 1	Month 3
1	1 89	0 34	0 05	0 19	91	209	189
2	0 68	0 20	0 50	0 39	186	85	109
4	0 39	1 32	0 78	0 79	168	149	146
5	1 05	1 16	0 29	1 15	184	129	154
6	0 54	0 41	0 26	0 14	68	108	66
7	2 16	0 50	1 89	1 49	162	158	154
8	1 03	0 73	1 23	0 70	188	76	80
9	N A	1 08	0 38	0 92	79	103	93
10	0 97	0 95	1 35	0 50	215	167	270
11	0 65	0 46	0 55	1 28	190	198	180
13	0 47	0 20	0 28	0 15	146	70	91
14	0 65	0 68	0 42	0 59	165	149	149
15	0 30	0 26	0 27	0 28	179	189	214
16	1 04	0 90	1 17	1 47	216	214	244
18	1 46	1 03	N A	1 66	94	181	138
19	0 57	0 59	0 58	2 70	180	150	215
22	3 09	1 89	1 88	1 17	105	137	111
23	2 03	0 49	0 60	1 49	127	143	126
25	0 42	0 32	0 38	0 27	72	120	86
26	0 82	0 88	0 72	0 53	175	170	150
27	1 66	1 46	0 80	3 24	173	260	180
28	0 49	0 24	0 28	0 38	221	164	172
29	2 70	1 07	1 13	1 20	72	117	91
30	0 34	0 72	0 22	0 39	153	167	196
32	0 22	0 47	0 78	0 36	N A	229	209
33	0 31	0 09	0 61	0 43	103	162	152
34	1 65	1 86	N A	0 88	142	68	134
35	0 73	0 39	N A	1 23	192	164	204
7	0 62	0 41	0 34	0 37	139	116	148
	1 28	0 70	0 72	1 15	123	125	86
	1 13	1 04	0 90	0 30	175	214	170

DISCUSSION

This study confirms the antihypertensive effect of pindolol in essential hypertension (5 6 7 13 14 18) There was an unexpected rise of BP in a sub stantial proportion of the patients (16 out of 31) during the third month of pindolol treatment when the drug dose was at its highest This observation resembles that of Waal Manning and Simpson (17) who showed a reduction of BP in nine hypertensive patients treated with pindolol when the drug doses were lowered Bjerle et al (3) also reported that decreasing the dose of pindolol by 33% decreased the BPs in 20 patients with essential hypertension The slight rise of BP towards control levels during increasing doses of pindolol remains unexplained Drug doses did not differ between the responders and non responders although this would have been expected from the design of the study The reason for this may be that the antihypertensive effect of pindolol was in fact quite similar until the last month of drug treatment (Fig 1) It is emphasized that good antihypertensive control was obtained in the non responders while on the lower doses of pindolol (Fig 1)

The reduction of heart rate observed by us confirms previous data (4 5 16 17) The interesting observation that heart rate (upright position) rose in the non responders during the last month of drug treatment may have several explanations It is conceivable that some of these patients stopped taking their drug although none admitted to doing so This explanation cannot be excluded but is unlikely be cause pulse rate remained lower than after placebo and all patients returned their drug bottles at the end of the study with the correct number of pills left It would also be difficult to explain why these

Table IV *Plasma renin activity urinary and serum electrolytes serum creatinine haemoglobin and haematocrit values in the whole patient material*

	Placebo month	Pindolol treatment Month 1 (10 0 mg/day)	Month 2 (14 2 mg/day)	Month 3 (19 0 mg/day)
PRA (ng/ml h)	1 07±0 13	0 74±0 09**	0 69±0 09 **	0 90±0 13
Urinary sodium excretion (mmol/24 h)	149±8 4	151±8 5		152±9 3
Urinary potassium excretion (mmol/24 h)	69±3 6	60±4 6		60±3 8
Serum Na⁺ (mmol/l)	144±0 4			143±0.5
Serum K⁺ (mmol/l)	4 3±0 08			4 6±0 09*
Serum creatinine (μmol/l)	84±2 9			83 7±2 1
Hb (g/l)	146±2 7			150±2 1
Hct (%)	45 3±0 7			41 5±0 7*

Significance of changes compared with values at the end of placebo period * $p<0\ 05$ * $p<0\ 005$ ** $p<0\ 001$

patients having taken their drug regularly for two months as judged from BP and pulse data should suddenly stop taking it during the third month yet complain of no serious side effects Pindolol is known to decrease pulse rate less than the β adrenergic blockers with little or no intrinsic β mimetic activity e g propranolol (10) The increased pulse rate in some patients may be related to the intrinsic β mimetic effect of pindolol

The association of rising BP and PRA despite rising doses of pindolol does not necessarily imply a cause and effect relationship Such a relationship between antihypertensive and PRA lowering effect of β adrenergic blocking drugs has been claimed by some investigators (4 5 18) and denied by others (1 9 10) In this study changes in PRA and BPs were not significantly correlated This supports the idea that the antihypertensive effect of pindolol in essential hypertension is not related to suppression of PRA (2 9 10)

The male preponderance (2 females 14 males) in the group of therapeutic failures (Table II) was not coincidental because 14 women and 17 men completed the study There were no differences in duration or severity of hypertension between the two subgroups of antihypertensive response neither were there differences in age obesity electrolytes or ECG findings

PRA values were different during the last month of pindolol therapy (Fig 1) Smoking habits also differed Thus 12 men and one woman were cigarette smokers Of the smokers ten were considered therapeutic failures and the most unfavourable BP responses at the end of the study were recorded in the exceptional patients reporting heavy smoking (more than 50 cigarettes daily)

Male patients with essential hypertension appear to respond less well than female patients to pindolol monotherapy This may be due to sex differences to factors associated with smoking or to higher sensitivity to the intrinsic β mimetic activity of pindolol Patients with mild essential hypertension appear to maintain antihypertensive control with low doses 10–15 mg daily of pindolol The frequency of drug resistance to pindolol may increase with higher drug doses Lowering the dose should then be tried (16 18)

ACKNOWLEDGEMENTS

F F was supported by the Finnish National Research Council for Medical Science (1663 3) and the Sigrid Juselius Foundation Helsinki

REFERENCES

1 Amery A Billiet L & Fagard R. Beta receptors and renin release N Engl J Med 290 284 1974
2 Anavekar S N Louis W J Morgan T O Doyle A E & Johnston C I The relationship of plasma levels of pindolol in hypertensive patients to effects on blood pressure plasma renin and plasma noradrenaline levels Clin Exp Pharmacol Physiol 2 203 1975
3 Bjerle P Jacobsson K A & Agert G Adrenergic β receptor blockade in essential hypertension A comparison between pindolol (Visken) and propranolol (Inderal) Curr Ther Res 18 387 1975
4 Buhler F R Laragh J H Baer L Darracott Vaughan E Jr & Brunner H R Propranolol inhibi

Table III Individual values of plasma renin activity and sodium excretion
N A = not available

Pat no	PRA (ng/ml h) After placebo	Pindolol treatment Month 1	Month 2	Month 3	Urinary sodium excretion (mmol/24 h) After placebo	Pindolol treatment Month 1	Month 3
1	1 89	0 34	0 05	0 19	91	209	189
2	0 68	0 20	0 50	0 39	186	85	109
4	0 39	1 32	0 78	0 79	168	149	146
5	1 05	1 16	0 29	1 15	184	129	154
6	0 54	0 41	0 26	0 14	68	108	66
7	2 16	0 50	1 89	1 49	162	158	154
8	1 03	0 73	1 23	0 70	188	76	80
9	N A	1 08	0 38	0 92	79	103	93
10	0 97	0 95	1 35	0 50	215	167	270
11	0 65	0 46	0 55	1 28	190	198	180
13	0 47	0 20	0 28	0 15	146	70	91
14	0 65	0 68	0 42	0 59	165	149	149
15	0 30	0 26	0 27	0 28	179	189	214
16	1 04	0 90	1 17	1 47	216	214	244
18	1 46	1 03	N A	1 66	94	181	138
19	0 57	0 59	0 58	2 70	180	150	215
22	3 09	1 89	1 88	1 17	105	137	111
23	2 03	0 49	0 60	1 49	127	143	126
25	0 42	0 32	0 38	0 27	72	120	86
26	0 82	0 88	0 72	0 53	175	170	150
27	1 66	1 46	0 80	3 24	173	260	180
28	0 49	0 24	0 28	0 38	221	164	172
29	2 70	1 07	1 13	1 20	72	117	91
30	0 34	0 72	0 22	0 39	153	167	196
32	0 22	0 47	0 78	0 36	N A	229	209
33	0 31	0 09	0 61	0 43	103	162	152
34	1 65	1 86	N A	0 88	142	68	134
35	0 73	0 39	N A	1 23	192	164	204
	0 62	0 41	0 34	0 37	139	116	148
	1 28	0 70	0 72	1 15	123	125	86
	1 13	1 04	0 90	0 30	175	214	170

DISCUSSION

This study confirms the antihypertensive effect of pindolol in essential hypertension (5 6 7 13 14 18) There was an unexpected rise of BP in a sub stantial proportion of the patients (16 out of 31) during the third month of pindolol treatment when the drug dose was at its highest This observation resembles that of Waal Manning and Simpson (17) who showed a reduction of BP in nine hypertensive patients treated with pindolol when the drug doses were lowered Bjerle et al (3) also reported that decreasing the dose of pindolol by 33% decreased the BPs in 20 patients with essential hypertension The slight rise of BP towards control levels during increasing doses of pindolol remains unexplained Drug doses did not differ between the responders and non responders although this would have been expected from the design of the study The reason for this may be that the antihypertensive effect of pindolol was in fact quite similar until the last month of drug treatment (Fig 1) It is emphasized that good antihypertensive control was obtained in the non responders while on the lower doses of pindolol (Fig 1)

The reduction of heart rate observed by us con firms previous data (4 5 16 17) The interesting observation that heart rate (upright position) rose in the non responders during the last month of drug treatment may have several explanations It is con ceivable that some of these patients stopped taking their drug although none admitted to doing so This explanation cannot be excluded but is unlikely be cause pulse rate remained lower than after placebo and all patients returned their drug bottles at the end of the study with the correct number of pills left It would also be difficult to explain why these

Acta Med Scand 202 61-64 1977

Bullous Dermatosis among Patients with Chronic Renal Failure on High Dose Frusemide

Gerhard Heydenreich Torben Pindborg and Henning Schmidt

From the Departments of Dermatology and Nephrology
Odense University Hospital Odense Denmark

ABSTRACT Twelve of 56 patients with chronic renal failure all treated with frusemide (Lasix®) in daily doses of 0 5-2 g developed bullae in areas exposed to light In most cases the bullae developed during summer months and disappeared later in the year whether the frusemide treatment was continued or not In two patients the eruption reappeared when treatment was resumed Disturbance of the porphyrin metabolism was not found neither could a change in the frusemide metabolism be demonstrated Tissue typing and blood groups showed no difference from the average population It is concluded that the condition presumably is a photo reaction due to the frusemide treatment but it cannot be said whether it is allergic or toxic

In recent years we have observed a bullous skin disease among patients suffering from chronic renal failure All were treated with frusemide in doses up to 2 g daily Since we were not acquainted with the pathological picture and as it had not then been reported in the literature the cases were registered according to their clinical appearance as epidermolysis bullosa acquisita obs porphyria cutanea tarda The characteristic feature was that the blisters appeared on clinically normal skin and mostly in areas exposed to light or trauma

Korting (8) was the first to report two cases of a similar skin disease among patients undergoing chronic hemodialysis He demonstrated an increased secretion of porphyrins in the urine of one patient and therefore believed the diagnosis to be porphyria cutanea tarda Kennedy and Lyell (7) have later reported seven cases described as epidermolysis bullosa acquisita which they believed was caused by frusemide in high doses given to patients with chronic renal failure

In the papers mentioned here the morphological changes are identical with those observed by us in patients with chronic renal failure treated intensively with frusemide

PATIENTS

In 1973-75 56 patients (29 men and 27 women) suffering from chronic renal failure were treated with frusemide (Lasix®) in daily doses of 0 5-2 g Almost all patients were undergoing chronic hemodialysis The high frusemide doses were given mainly to prevent fluid retention but also to allow a more liberal liquid consumption Twelve of the patients (5 women and 7 men aged 25-69 years) showed bullous skin changes during the spring and summer mainly localized to hands and feet

The underlying renal disease is shown in Table I Six of the patients were dialyzed at the hospital centre three at home and three were in the predialytic phase two of whom had been kidney transplanted Various medicines had been taken before the bullous changes appeared Frusemide however had been given constantly to all patients in daily doses of 0 5-2 g during a period of at least one month before the eruptions Three patients had received no medicine except frusemide The patients were heparinized during dialysis Eight patients had not previously suffered from skin diseases two had suffered from herpes zoster one from congenital ectodermal dysplasia and one from keratotic eczema During their uremic phases all patients suffered from pruritus They all developed bullous eruptions on the dorsal surfaces of hands and fingers (Fig 1) Three patients also had bullous eruptions on the dorsa of feet and toes three developed minor bullae in the face and two had bullae on the neck and upper trunk All patients developed bullae on clinically normal skin The bullae contained serous fluid in one patient however the fluid was temporarily hemorrhagic In one case the bullous eruption was accompanied by pruritus The blisters healed without scarring or milia

In all patients the bullous eruptions started during May-Sept i e the most sunny months Several patients were keen on sunbathing and were very tanned but had bullous

Table I *Clinical data on the 12 patients developing bullous skin changes*

Pat no	Age (y)	Sex	Diagnosis	Lasix dose (g/d)	Duration of treatment before bullous eruption (mo)	Bullous eruptions started	Location of bullae
1	50	♀	Renes polycystici	1	12	June 1975	Fingers toes crura forearms upper trunk
2	58	♀	Nephropathia causa ignota	½	12	June 1975	Dorsa of hands forearms, face
3	69	♂	Nephrosclerosis	2	3 16	May 1975 June 1976	Dorsa of hands forearms ears
4	47	♀	Glomerulonephritis chronica	1	3	June 1975	Dorsa of fingers and toes
5	50	♂	Pyelonephritis chronica	1	3	Sept 1975	Dorsa of hands forearms
6	25	♀	Nephropathia congenita	1	9	July 1975	Dorsa of hands nose
7	65	♂	Glomerulonephritis chronica	1	1	May 1974	Dorsa of hands fingers
8	49	♂	Renes polycystici	½	5	April 1974	Dorsa of hands
9	51	♀	Pyelonephritis chronica	2	12	Aug 1974	Dorsa of hands fingers
10	58	♂	Nephropathia diabetica	1	11	May 1975	Dorsa of hands dorsa of feet
11	25	♂	Glomerulonephritis chronica	2	5 16	Aug 1975 July 1976	Dorsa of hands
12	38	♂	Lupus erythematosus disseminatus	¾	5	July 1976	Dorsa of hands

ons only in areas generally exposed to light i e of hands and feet and in the face Unlike patients fering from porphyria cutanea tarda no hypertrichosis coarsed skin was seen among these patients The bullae disappeared spontaneously after varying intervals whether the frusemide treatment was continued or not Two patients who showed no symptoms after discontinuation of frusemide treatment developed new bullous eruptions after frusemide had been resumed These eruptions however developed during Oct and Dec One of these patients was given i v frusemide owing to pulmonary edema and the bullous eruptions recurred within 24 hours of resuming the treatment Two patients who received frusemide continuously developed bullae during two consecutive summers

METHODS

Immunofluorescence studies were carried out as previously described (5) The skin biopsies were examined for presence of immunoglobulins (IgG IgA IgM) and complement (C_3) fixed in vivo to the basement membrane or to the intercellular substance in epidermis Sera were examined for presence of circulating basement membrane antibodies and epidermal intercellular substance antibodies

Porphyrin studies were carried out in erythrocytes and plasma as a thin layer chromatography according to the method described by With (9) Also a qualitative urine screening was made

To determine the serum frusemide concentration a fluorometric method was used (3)

RESULTS

Light microscopy showed subepidermal bulla formation with sparse mononuclear cell infiltration in the underlying corium Immunofluorescence studies of affected and normal skin from two patients did not show any in vivo fixed or circulating antibodies to the basement membrane or the intercellular substance in epidermis Bacteriological investigations showed the bullous fluid to be sterile

Porphyrin studies in erythrocytes and plasma from seven patients with bullae showed the same results as seven dialysis patients without bullae Urine screening was negative in eight patients.

Serum frusemide was determined in five patients with bullae and in nine hemodialysis patients w



Fig 1 Large tense bullae on exposed skin

out bullae No significant difference was found be tween the two groups

Tissue typing was made in ten patients blood group determination in 12 (Table II) No deviations from the average population were found

All patients had normal liver function tests Scratch and patch tests with frusemide 5% in petrolatum were made on two patients the results were negative No photo patch testing was made

DISCUSSION

Korting (8) believed that porphyrins caused the bullous eruptions in patients undergoing mainte nance hemodialysis since porphyrin excretion was found in the urine of one patient In the present paper dealing with 12 patients the porphyrin in vestigations on erythrocytes and plasma were nor mal and the urine screening negative This proves that porphyrins are not responsible for this disease Nor have other authors (1 4 7) been able to prove the influence of porphyrins on this pathological picture Light microscopy and immunofluorescence studies showed only non specific changes

Serum frusemide was found to be similar in pa tients with and without bullae Bulla formation in one group therefore, cannot be due to an abnormal ity in the frusemide metabolism Patients suffering from bulla formation did not differ from the average population as regards tissue type antigens or blood groups Frusemide patch tests and scratch tests did not detect any sensitization towards frusemide

Phototoxic reactions after administration of sul phanilamide have long been known (2) Antidia betics of the sulphonylurea group as well as thiazides and frusemide are derivatives of sulpha nilamide There are several reports on antidiabetics and thiazides as a cause of photo reactions (6) but it is only during the last year that photo reactions presumably due to frusemide have been reported (1) This is probably because high frusemide doses have only been given to hemodialyzed patients in recent years

Among our patients the bullous eruptions devel oped during sunny months whereas Burry and Lawrence (1) from South Australia did not find any seasonal variations This is probably due to the dif ference in light intensity between the two countries Gilchrest et al (4) believed that the chronic hemo dialysis itself caused the bullous eruptions They suspected a phototoxic reation but were not able to produce an experimental bullous eruption by using monochromatic light

In the present paper 12 out of 56 patients suffer ing from chronic renal failure and treated with frusemide in doses up to 2 g daily developed bullous eruptions in areas exposed to light On the basis of this material we suggest that the high doses of frusemide and not the hemodialysis make the pa tients hypersensitive to light We believe that the

Table II *Tissue typing and blood groups*

Pat no	HLA types (HLA A and HLA B loci)	Blood group
1	A3 9 B12 w40	O−
2		O+
3		A+
4	A1 9 Bw40	A+
5	A28 w19 B5 18	AB+
6	A1 11 B8 w17	O+
7	A9 w25 B5 18	B+
8	A1 9 B7 w16	O+
9	A10 B8 w21	B+
10	A1 w33 Bw40 w40	O+
11	A2 Bw15 w35	O+
12	A2 3 B12 w40	O+

hemorrhagic bullae found in one patient were due to simultaneous heparinization during hemodialysis We are not in a position to conclude anything about the nature of the photo reactions

REFERENCES

1 Burry J N & Lawrence J R Br J Dermatol 94 495 1976
2 Epstein S J Invest Dermatol 2 43 1939
3 Forrey A W Kimpel B Blair A D & Cutler R E Clin Chem 20 152 1974
4 Gilchrest B Rowe J W & Mihm M C Ann Intern Med 83 480 1975
5 Heydenreich G From E & Diederichsen H Acta Derm Venereol (Stockh) 52 201 1972
6 Jarratt M Int J Dermatol 15 317 1976
7 Kennedy A C & Lyell A Br Med J 2 1509 1976
8 Korting G W Dermatologica 150 58 1975
9 With T K Dan Med Bull 22 74 1975

Acta Med Scand 202 65–67, 1977

Renal Glucosuria and Aminoaciduria

Ole Gøtzsche

From Second University Clinic of Internal Medicine Kommunehospitalet Århus Denmark

ABSTRACT A follow up examination of five patients in whom renal glucosuria had been diagnosed 7–15 years previously, showed that the condition was unchanged There was no indication of hormonal abnormalities Oral glucose tolerance test, with determination of insulin, growth hormone and free fatty acids, showed no difference between the patients and a group of normal subjects The urinary excretion of insulin and albumin was normal, but two patients turned out to have an increased excretion of certain amino acids, aspartic acid in one and glutamic acid, citrulline and alanine in the other

Renal glucosuria (RG) is usually defined as an inherited abnormality with constant glucosuria a normal 24-hour blood glucose level and a normal glucose tolerance

A familial occurrence of glucosuria with aminoaciduria has previously been described (6) and Elsas et al (3) found high excretion of two amino acids in a patient with RG No report has appeared on albumin excretion in RG

A number of previous investigations of RG have indicated that this condition has no relation to diabetes mellitus especially not to classical juvenile diabetes (7) However the existence of a so-called mild juvenile diabetes has now been established This type of diabetes is best defined as a disease occurring in young non-obese persons in whom the 24-hour blood glucose is slightly but significantly increased There are no or only very mild symptoms There is no ketonuria and treatment with diet and/or antidiabetic drugs is effective These patients have low plasma insulin response during a glucose tolerance test quite different from the initial hyporesponse followed by high plasma insulin values seen in maturity onset diabetes (5) Recently three families with several such persons have been described (14) Glucosuria at a low blood glucose level was found in a number of these persons

The purpose of this communication is to report the results of a series of hormonal and renal examinations in patients who have had RG for several years The glucose metabolism has been estimated by an oral glucose tolerance test with determination of plasma insulin serum growth hormone and serum free fatty acids In addition in order to examine the tubular defect the urinary excretion of insulin albumin and amino acids was determined

PATIENTS AND METHODS

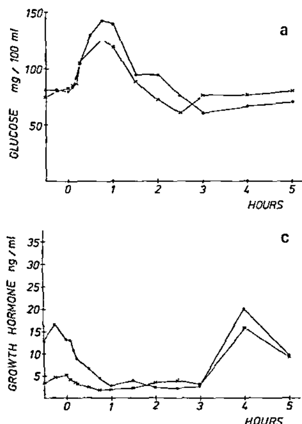
Five patients (three males and two females) were selected according to the following criteria Young (i e under 30 years) otherwise healthy and non obese persons in whom the diagnosis of RG had been made 7–15 years previously They had constant glucosuria also in the fasting state and the oral glucose tolerance test had shown normal results They all had near relatives with RG

For the oral glucose tolerance test the patients arrived at the hospital in the morning in the fasting state After they had rested in bed for half an hour blood was collected via an i v catheter 30 min later the patients drank 70 g glucose dissolved in 250 ml water and during the following five hours 5 ml blood samples were collected via the catheter EDTA was added to the blood which was centrifuged and stored at −20°C until the hormone analysis Urine was sampled every hour and stored at −20°C for glucose and insulin determination

In order to examine the glucose and albumin excretion in the fasting state the patients collected the urine from 8 to 12 a m on two mornings after overnight fasting A 24-hour sample for determination of amino acid excretion was obtained some months later

For comparison five normal non-obese and age matched persons underwent the same examinations

Plasma glucose was determined by a glucose oxidase method (1) and urinary glucose by the paratoluidin method Plasma insulin urinary insulin and serum growth



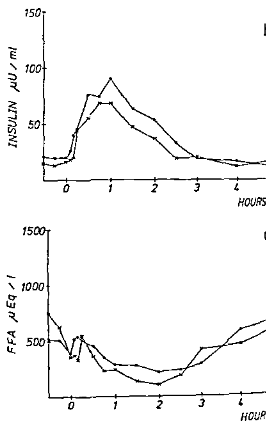


Fig 1 Oral glucose tolerance test Median values of blood glucose (*a*) plasma insulin (*b*) serum growth hormone (*c*) and serum free fatty acids (*d*) in five patients with renal glucosuria (o) and in five normal subjects (×) Glu intake at time 0

estimated by radioimmunoassay employing -chromatography (16 17) Free fatty acids were de by a modified radiochemical method (4) For the estimation of albumin excretion we used a radioimmunoassay with single antibody (8) The amino acid excretion in 24-hour urine sample was determined quantitatively by column chromatography

Statistical evaluations were carried out with the non parametric Wilcoxon test

RESULTS

The glucose tolerance tests were normal like those carried out 7–15 years previously Fig 1*a* shows the median values for the five patients and the five normal subjects The curves are identical

The glucose excretion in the fasting state is still high (Table I) The plasma insulin curves are identical (Fig 1*b*) Only one pair of estimations shows a statistical difference at the 5% level (2 1/2 hour value) but all values are clearly normal The serum growth hormone curves (Fig 1*c*) show no difference ($p>0\ 10$) The apparent divergence in the beginning was due to the fact that the values in the two women with RG were on their way down fro spike just before the glucose intake The concen tions of free fatty acids were identical in the groups ($p>0\ 05$) (Fig 1*d*)

The insulin clearance estimated as a mean du the glucose tolerance test, showed no signifi difference ($p>0\ 10$) between the two gro (Table I)

Table I *Urinary excretion in patients with re glucosuria*

	Pat no				
	1	2	3	4	5
Glucose excretion in the fasting state (mg/min)	1 67	0 55	0 40	0 27	[illegible]
Albumin excretion* (μg/min)	13 0	4 7	3 5	4 3	[illegible]
Insulin clearance (ml/min)	0 48	0 27	0 63	0 66	[illegible]

* Normal subjects mean 8 7 μg/min S D 8 0 (9)

Table II *Amino acids (µmol/24 h) excreted in excess in 24 hour urine from two patients with renal glucosuria*

	Pat no		Upper normal limit
	2	3	(10 13)
Aspartic acid	226	–	218
Glutamic acid	–	87	44
Citrulline	–	14	6
Alanine	–	564	494

The quantitative determination of 24 amino acids in a 24 hour specimen of urine carried out in the five patients showed that three had an excretion of certain amino acids (Table II) that exceeded the upper normal limit (10 13) However one of these patients was pregnant when the urine collection took place

DISCUSSION

The examination of five patients who had had RG for 7–15 years selected according to the criteria indicated by Marble (7) showed that the condition had remained stationary None of the patients had developed mild juvenile diabetes or any other form of diabetes Plasma insulin during the glucose tolerance test was normal The normal increase in serum growth hormone occurred at the expected time after glucose administration without the early increased values found in diabetes mellitus (2 15) During the increase in plasma insulin the concentration of free fatty acids was suppressed equally in the two groups

Insulin is filtered in the glomeruli reabsorbed and decomposed in the proximal tubuli which is the location of reabsorption of glucose (12) Clearance determination revealed no sign of abnormal insulin reabsorption and/or breakdown in the kidney of patients with RG

Increased excretion of amino acids was found in three patients one of whom was a pregnant woman (Table II) Fanconi s syndrome is characterized by a general insufficiency of the reabsorptive processes in the proximal tubuli This may lead to glucosuria unspecific aminoaciduria hyperphosphaturia hyperpotassuria and possibly other reabsorption defects The clinical symptoms are osteomalacia and muscle weakness (11) Our two patients cannot be classified as suffering from Fanconi s syndrome because the aminoaciduria was only partial because there was no albuminuria (Table I) and because none of them had clinical symptoms of this disease The same is true of their relatives with RG

A familial occurrence of glucosuria combined with aminoaciduria without the other signs of Fanconi s syndrome has previously been described (6) The tubular defect was shown in three successive generations and the aminoaciduria showed the same pattern in all patients Proline leucine and valine were excreted in high concentrations There were no clinical symptoms Elsas et al (3) also found a mild aminoaciduria (alanine leucine) in one of their patients with RG Our two patients with RG (both male) did not differ from the other three with respect to weight age glucose tolerance albuminuria or urinary insulin excretion It is not possible to deduce anything from the third case the pregnant woman because aminoaciduria occurs often during pregnancy

The present study confirms that in some cases RG may be characterized by tubular abnormality not only of the reabsorption of glucose but also of that of certain amino acids Further investigation of the different tubular functions in patients suffering from RG may disclose different types perhaps with different patterns of inheritance

REFERENCES

1 Christensen N J Scand J Clin Lab Invest 19 379 1967
2 Christensen N J Ørskov H & Hansen Å P Acta Med Scand 192 337 1972
3 Elsas L J Busse D & Rosenberg L E Metabolism 20 968 1971
4 Ho R J & Meng H C Anal Biochem 31 426 1969
5 Johansen K Acta Med Scand 193 23 1973
6 Luder J & Sheldon W Arch Dis Child 30 160 1955
7 Marble A Joslin s diabetes mellitus p 818 Lea and Febiger Philadelphia 1971
8 Miles D W Mogensen C E & Gundersen H J G Scand J Clin Lab Invest 26 5 1970
9 Mogensen C E & Vittinghus E Scand J Clin Lab Invest 35 295 1975
10 Moore S & Stein W J Biol Chem 211 907 1954
11 Mudge G H Am J Med 24 785 1958
12 Rubenstein A H & Spitz I Diabetes 17 161 1968
13 Soupart P Clin Chim Acta 4 265 1959
14 Tattersall R B Q J Med 43 339 1974
15 Yde H Acta Endocrinol 64 339 1970
16 Ørskov H Thomsen H G & Yde H Nature 219 193 1968
17 Ørskov H & Johansen K Acta Endocrinol 71 697 1972

Acta Med Scand 202 69–73 1977

Screening for and Treatment of Bacteriuria in a Middle-aged Female Population

I The Prevalence of Bacteriuria Urinary Tract Infections under Treatment and Symptoms of Urinary Tract Infections in the Säkyla Köyliö Project

Jorma Takala Hannele Jousimies and Kai Sievers

From the Department of Public Health University of Turku Turku and Central Public Health Laboratory Helsinki Finland

ABSTRACT In connection with the screening programme of the Sakyla Köylio project, the prevalence of untreated bacteriuria, urinary tract infection (UTI) under treatment and UTI symptoms has been investigated among the middle aged (40–64 years) female population (*n*=1312) of the Sakylä and Koylio municipalities 93 2% of the total population participated in the screening The Uricult® dip slide was used in the quantitative urine culture Before collection of the urine specimens, the subjects were interviewed about symptoms of UTI and use of related medications The total prevalence of untreated bacteriuria among the women was 4 5%, asymptomatic bacteriuria accounting for the majority of cases (82%) This prevalence increased with age, tripling in the oldest age group A total of 2 5% of the women were being treated for UTI at the time of the examination Thus the combined prevalence of untreated bacteriuria and UTI under treatment was 7% Altogether 6 4% of the women had symptoms of UTI, with an almost equal distribution among the age groups The total prevalence of symptoms of UTI without actual UTI (treated and untreated bacteriuria) was 5 0% Every fourth woman with dysuria had been treated for UTI during the previous year and about every third at some time earlier than that This frequency suggests an etiology of UTI for the discomfort

Bacteriuria is the most common denominator of urinary tract infections (UTI) (18) while pyelonephritis the severest form of UTI is the most common kidney disease and a major cause of renal failure (17 23) Persons with bacteriuria are regarded as potential pyelonephritis patients because pyelonephritis is thought to originate often from bacteriuria (14) In addition asymptomatic bacteriuria greatly increases the risk of pyelonephritis in pregnant women and an active screening for and treatment of bacteriuria can definitely reduce this risk (16 20)

Ever since the success of screening for bacteriuria among pregnant women was ascertained investigations have increasingly been directed toward non pregnant women However very few investigations on the success of screening for and treatment of bacteriuria have involved unselected middle aged populations Moreover studies on the prevalence of bacteriuria have seldom dealt with the prevalence of UTI under treatment and symptoms of UTI Therefore our aim was to treat the screening for and treatment of bacteriuria in an unselected middle aged female population in two reports the present article on prevalences of untreated bacteriuria UTI under treatment and symptoms of UTI and another on the feasibility of such a screening programme

SUBJECTS AND METHODS

The subjects consisted of the middle aged (40–64 years) female population of the Säkyla and Koylio municipalities (*n*=1312) A total of 1223 (93 2%) of the women participated in our screening programme that went on from Sept 9 1973 to Jan 11 1974 (32)

Collection of urine specimens

Specimens of mid-stream urine which had been in the bladder for at least 4 hours (31) were collected in a clean disposable container Instructions emphasising thorough cleansing of the external genitals were enclosed in the invitation to participate Before collecting the specimen

Table I *Prevalence of untreated bacteriuria*

Age group	No of subj	Asymptomatic bacteriuria		Symptomatic bacteriuria		Total	
		N	%	*N*	%	*N*	%
40–49	561	11	2 0	3	0 5	14	2.5
50–59	450	20	4 4	3	0 7	23	5 1
60–64	212	14	6 6	4	1 9	18	8 5
Total	1 223	45	3 7	10	0 8	55	4.5

the nurse responsible for the screening programme asked about symptoms of UTI (dysuria and/or increased fre quency of urination) and about any possible medication for UTI She also instructed each subject individually on how to collect the specimen The instructions published by Orion Diagnostica were followed except that physiological saline was used for the cleansing instead of a soap solution

Quantitative bacterial culture of urine

The specimens were immediately cultured in the local laboratory according to the dip slide method (Uricult® Orion Diagnostica) and incubated at +37 C overnight On the following day the dip-slides were examined according to the manufacturer s instructions Clearly negative slides were discarded and the others (>10^3 bacteria/ml urine) were sent to the Central Public Health Laboratory Helsinki where re examination and final bacterial count were carried out In cases of significant (>10^5 bacteria/ml) (12) and doubtful (10^4–10^5 bacteria/ml) findings sub cultures and bacterial identifications were made with standard procedures (6 15) Sensitivity testing on the most common UTI chemotherapeutics were carried out by the agar diffusion method (7) Lactose fermenting and ... ve rods with typical morphological and growth characteristics were classified from the dip slides as coli forms

Interpretation of results of culture

Control specimens were taken after about 2 weeks when the following conditions were met (*a*) more than 10^5 bacteria/ml but no symptoms of UTI (*b*) more than 10^3 but less than 10^5 bacteria/ml and a pure culture of a single bacterium or (*c*) less than 10^5 bacteria/ml and simultane ous symptoms indicative of UTI Cases in which more than 10^5 bacteria/ml of the same bacterial strain (13) grew in two consecutive specimens were classified as asymptomatic bacteriurias In symptomatic cases we considered one positive (>10^5) finding to be sufficient for the diagnosis of bacteriuria in agreement with some other investigators (4 24 31)

RESULTS

Prevalence of untreated asymptomatic and symptomatic bacteriuria

More than 10^5 bacteria/ml were found in the first specimen from 50 untreated asymptomatic women The second specimens from five of them no lon[ger] had such a high bacterial level Consequently (10%) out of 50 first specimens were falsely po[si]tive or showed a spontaneous recovery Ten sym[p]tomatic untreated cases were diagnosed from [a] single specimen, 8 with more than 10^6 and 2 w[ith] 10^5–10^6 bacteria/ml

Table I shows the prevalences of both asy[mp]tomatic and symptomatic bacteriuria Untrea[ted] bacteriuria was present in 4 5% of the women T[he] prevalence increased with age being in the old[est] age group more than threefold and in the mid[dle] group twofold, that of the youngest age gr[oup] ($\chi^2_{(2)}$=13 50 p<0 01) Asymptomatic bacteri[uria] was involved in the majority of cases (82%) P[ro]teus mirabilis grew in one specimen only (2[%]) coliforms were found in all the others ... nitrofurantoin resistant strain was present in th[e] ... cases

Prevalence of UTI under treatment

Table II depicts the prevalence of UTI which w[as] 7% of the entire material (untreated bacteriu[ria] + UTI under treatment) In the oldest age gr[oup] this prevalence was twofold that of the young[est] age group ($\chi^2_{(2)}$=7 84 0 01<p<0 02) At the time [of] the examination 2 5% of all the women were bei[ng] treated for UTI which was checked from their p[re]scriptions The urinary finding of two of these wo[m]en (one Proteus mirabilis and one coliform) w[as] more than 10^5 bacteria/ml Relatively more of t[he] subjects in the youngest age group (every secon[d]) than in the middle (every third) or oldest age gro[up] (every sixth) were being treated for UTI Twen[ty] four (77%) of those receiving therapy had sym[p]toms at the start of treatment

Prevalence of symptoms of UTI

Symptomatic patients were divided into two group[s] according to whether they had UTI (both treate[d] and untreated) or not (Table III) A total of 6 4% (

Table II *Prevalence of treated and untreated bacteriuria*

Age group	No of subj	Untreated bacteriuria		UTI under treatment		Total	
		N	%	N	%	N	%
40–49	561	14	2.5	14*	2.5	28	5 0
50–59	450	23	5 1	13	2 9	36	8 0
60–64	212	18	8 5	4	1 9	22	10 4
Total	1 223	55	4 5	31	2 5	86	7 0

In three cases the treatment had been commenced on the basis of symptoms and no urine specimen had been collected

all the women had symptoms equally distributed among the age groups Altogether 5% of all the women had UTI symptoms without having bacteriuria or without being treated for UTI at the time of examination One fourth (26%) of these women had received UTI medication during the previous year and more than one third (36%) had been treated earlier

DISCUSSION

Possibility of false positive and false negative findings

The dip slide type of urine culture (5 10) has proved to be a reliable and suitable method for both clinical use and screening programmes (2 11 33) The results are most reliable when the dip-slides are first incubated at +37 C overnight and then sent to the bacteriological laboratory especially if a delay of several days is likely before analysis (1) This method was applied in the present study

Apparently because of the culture conditions and the accurate instructions given to the patients only 10% of the findings among asymptomatic women were falsely positive We were therefore able to make a reliable diagnosis of bacteriuria on the basis of two positive specimens (5) For the symptomatic subjects one positive finding was regarded as sufficient In the majority of these cases (8 out of 10) more than 10^6 bacteria/ml were present and this level is high enough to demonstrate UTI on the basis of only one culture (21) Less than 10^6 bacteria/ml (10^5–10^6) were found in 2 cases in both however the clinical symptoms clearly indicated UTI

Studies on the reliability of urine cultures have shown that an increased urination frequency (27) antibacterial medications (31) and the detergent used when collecting the specimens (30 31) may cause false negative results In our study only one of the 61 women with an increased frequency of urination and/or dysuria and a culture finding of less than 10^5 bacteria/ml in the first specimen had more than 10^5 bacteria/ml in the second specimen Moreover since a second specimen was collected 1 week after termination of medication from those who received antibacterial medication for any disease other than UTI at the time of the first specimen and since only physiological saline was used for the cleansing it is unlikely that any of these factors caused false negative results in our study

Bacteriuria and UTI under treatment

The total prevalence of bacteriuria in this study was about the same as in several reports from other

Table III *Prevalence of symptoms of UTI (dysuria and/or increased urination frequency)*

Age group	No of subj	Subj with symptoms and with bacteriuria or under treatment		Subj with symptoms but no bacteriuria or treatment		Total of subj with symptoms	
		N	%	N	%	N	%
40–49	561	5	0 9	29	5 2	34	6 1
50–59	450	8	1 8	22	4 9	30	6 7
60–64	212	4	1 9	10	4 7	14	6 6
Total	1 223	17	1 4	61	5 0	78	6 4

Table IV *Prevalence of bacteriuria found in some studies on adult female populations*

Age group	Bacteriuria (%) Rural	Bacteriuria (%) Urban	Ref no
15–24	0 8	1 9	25
25–34	4 4	1 0	Jamaica
35–44	5 0 } 4 9	1 8 } 2 2	
45–54	6 1	4.8	
55–64	11 8	1.8	
–20		0 8	8
20–29		1 0	Japan
30–39		1.8	
40–49		3 1 } 3 2	
50–59		2 8	
60–69		7 4	
70–		10 8	

Age group	Bacteriuria (%) Working women White	Working women Black	Nuns White	Nuns Black	Ref
15–24	4 8	9 0	0 4	1 1	19
25–34	3 6	8 1	0.3	0 0	US
35–44	5 0 } 4 4	5 1 } 7 4	1 2 } 0 9	1 2 } 1 6	
45–54	3 2	8 5	1 6	1 5	
55–64	6 6	4 5	2 8	5 4	
65–	7 4	(0)	6 1	3 9	
15–19	1.31				11
20–29	1.20				Fin
30–39	1 19				
40–49	1 88 } 2 31				
50–59	4 02				
60–69	6 02				
70–	4 76				
25–35	2 5				3
36–45	3 7				Swe
46–55	3 9 } 4 4				
56–65	3 9				
66–75	8 0				
75–	10 0				

countries but twice that found in another study on a Finnish female population (11) (Table IV) The reason for the low prevalence in the latter study have been the spontaneous cure that is quite in patients with UTI (22) since the inter between the two specimens was 2 months

Usually the results of screening for bacteriuria do not reveal the number of UTIs being treated The total number of UTIs is considerably underestimated when only bacteriuria is considered however it is difficult to establish with certainty how many of those being treated are receiving therapy for actual UTI and how many for symptoms only In our study when those being treated were included the total prevalence of UTIs was 7% Since only 10% of the women were being treated merely on the basis of symptoms i e without urine cultures the diagnosis of UTI can be considered valid in the majority of cases In addition almost half of the women being treated were receiving their medication free of charge because of chronic UTI

Symptoms of UTI without bacteriuria

Symptoms of UTI are very common among women On the basis of interviews it has been determined that almost 22% of the women aged 2(years experience dysuria during the year prio examination and almost half of these same wo at some other time in their lives (34) Althougl significant bacteriuria was found in about half of patients complaining of urinary discomfort to g eral practitioners a considerable number of th patients developed bacteriuria during the follov months (9) Therefore, the etiology of the urir discomfort in these cases was assumed to be U and these patients were thought to be at a st between two episodes of UTI (28) When the n stream specimen is free from bacteria urethritis be the cause of urinary discomfort (26) or it car caused by numerous other pathological conditi of the urinary tract or the genitals not associa with UTI (29) When no somatic cause for symptoms is found, it is assumed that the etiol factor is psychogenic (35) In the present study found symptoms of UTI without bacteriuria in of the women Since one fourth of these wom according to their own statements had been trea for UTI during the previous year and more tl one third of them at some time earlier there reason to assume that in many cases the etiolog the symptoms is somehow associated with UTI

REFERENCES

1 Alwall N Factors affecting the reliability of screening tests for bacteriuria II Dip-slide False positive results following postal transport and false negatives owing to incubation at room temperature Acta Med Scand 193 505 1973

2 Arneil G C McAllister T A & Kay P Detection of bacteriuria at room temperature Lancet 1 119 1970

3 Brandberg Å Hemer B Swartling P G & Vahlne G Urinvägsinfektion hos den vuxna befolkningen i en svensk stad Läkartidningen 67 2655 1970

4 Brumfitt W & Reeves D S Recent developments in the treatment of urinary tract infection J Infect Dis 120 61 1969

5 Cohen S & Kass E H A simple method for quantitative urine culture N Engl J Med 277 176 1967

6 Cowan S T & Steel K J Manual for the identification of medical bacteria Cambridge University Press Cambridge 1965

7 Ericsson H M & Sherris J C Antibiotic sensitivity testing Report of an international collaborative study Acta Pathol Microbiol Scand (A) (Suppl) 217 1 1971

8 Freedman L R Phair J P Seki M Hamilton H B Nefzger M D & Hirata M The epidemiology of urinary tract infections in Hiroshima Yale J Biol Med 37 262 1965

9 Gallagher D J A Montgomerie J Z & North J D K Acute infections of the urinary tract and the urethral syndrome in general practice Br Med J 1 622 1965

10 Guttmann D & Naylor G R E Dip-slide An aid to quantitative urine culture in general practice Br Med J 3 343 1967

11 Heinonen O P Laitinen H Takkunen H Jahkola M Virkola P & Makela P Bakteriurian diagnosointi aluslasimenetelmalla vaestötutkimuksessa Duodecim 85 1269 1969

12 Kass E H Asymptomatic infections of the urinary tract Trans Assoc Am Physicians 69 56 1956

13 — Bacteriuria and the diagnosis of infections of the urinary tract. With observations on the use of methionine as a urinary antiseptic Arch Intern Med 100 709 1957

14 — Renal and urinary disorders In Preventive medicine (ed D W Clark and B MacMahon) pp 539–552 Little Brown and Co Boston 1967

15 Kauffman F The bacteriology of Enterobacteriaceae Munksgaard Copenhagen 1966

16 Kincaid Smith P & Bullen M Bacteriuria in pregnancy Lancet 1 395 1965

17 Kleeman C R Hewitt W L & Guge L B Pyelonephritis Medicine 39 3 1960

18 Kunin C M Epidemiology of bacteriuria and its relation to pyelonephritis J Infect Dis 120 1 1969

19 Kunin C M & McCormack R C An epidemiologic study of bacteriuria and blood pressure among nuns and working women N Engl J Med 278 635 1968

20 Little P J The incidence of urinary infection in 5000 pregnant women Lancet 2 925 1966

21 Mabeck C E Studies in urinary tract infections I The diagnosis of bacteriuria in women Acta Med Scand 186 35 1969

22 — Treatment of uncomplicated urinary tract infection in non-pregnant women Postgrad Med J 48 69 1972

23 MacDonald R A Levitin H Mallory G K & Kass E H Relation between pyelonephritis and bacterial counts in the urine An autopsy study N Engl J Med 256 915 1957

24 Marsh F P Murray M & Panchamia P The relationship between bacterial cultures of the vaginal introitus and urinary infection In Urinary tract infection (ed W Brumfitt and A W Asscher) pp 178–185 Oxford University Press London 1973

25 Miall W E Kass E H Ling J & Stuart K L Factors influencing arterial pressure in the general population in Jamaica Br Med J 2 497 1962

26 Moore T & Hira N R The role of the female urethra in infections of the urinary tract. Br J Urol 37 25 1965

27 O Grady F Gauci C L Watson B W & Hammond B In vitro models simulating conditions of bacterial growth in the urinary tract. In Urinary tract infection (ed F O Grady and W Brumfitt) pp 80–90 Oxford University Press London 1968

28 O Grady F W Charlton C A C Fry I K McSherry A & Cattell W R Natural history of intractable cystitis in women referred to a special clinic In Urinary tract infection (ed W Brumfitt and A W Asscher) pp 81–91 Oxford University Press London 1973

29 Oravisto K J Female aseptic dysuria Ann Chir Gynaecol Fenn 61 295 1972

30 Roberts A P Robinson R E & Beard R W Some factors affecting bacterial colony counts in urinary infection Br Med J 1 400 1967

31 Suomen Mikrobiologit Virtsatieinfektioiden bakteriologinen tutkimus Suom Laak L 27 1311 1972

32 Takala J Sievers K Takala A & Karjalainen Y Säkylä Köyliö-projekti Terveyskeskustasolla toteutettava joukkotarkastus- hoito- ja seurantakokeilu I Tutkimuskohteet menetelmat ja osallistuminen joukkotarkastukseen Sos Laaket Aikak L 1 344 1975

33 Vejlsgaard R & Justesen T Quantitative bacterial culture of urine II Evaluation of 10 different screening methods for the diagnosis of bacteriuria compared with results obtained by the dilution technique Acta Med Scand 193 147 1973

34 Waters W E Prevalence of symptoms of urinary tract infection in women Br J Prev Soc Med 23 263 1969

35 Zufall R Treatment of the urethral syndrome in women JAMA 184 138 1963

Acta Med Scand 202 75–79 1977

Screening for and Treatment of Bacteriuria in a Middle-aged Female Population

II *Results of Short term Nitrofurantoin Therapy and One year Follow up*

Jorma Takala Hannele Jousimies and Kai Sievers

From the Department of Public Health University of Turku Turku and Central Public Health Laboratory Helsinki Finland

ABSTRACT Forty seven of the 55 subjects with bacteriuria found during a screening of an unselected female population aged 40–64 years (1312 women 93 2% of whom were examined) have been treated with a systematic 2 week nitrofurantoin therapy and 77% of them were cured During a 1 year follow up, urinary tract infection (UTI) recurred in 68% of those cured, thus only 24% of those treated had been cured and remained uninfected for a year Although the result of treatment in the group with no diabetes, hypertension or chronic rheumatoid arthritis was better (88%) than in the group with these diseases (46%), the cured subjects in both groups had a similar frequency of recurrences A previous UTI did not appear to have a negative effect on the result of treatment, neither did it increase the number of recurrent UTIs The fact that even in the group with no complicating diseases only 28% of those treated were cured and remained free from bacteriuria for 1 year proves that, so far no satisfactory therapy is available for mass use in the treatment of bacteriuria following a systematic screening of middle aged non pregnant women In addition to the lack of therapeutic methods, the systematic screening for bacteriuria in middle aged women was found to suffer from limited resources for further diagnostic examinations according to the present study, a total of 3% of middle aged women need further examinations following the screening for and treatment of bacteriuria

Opinions on screening for bacteriuria in non pregnant women are contradictory for its success has been both doubted (1) and supported (29) When evaluating a screening programme the possibilities of making further examinations and treating the disease to be screened are crucially important (36) So far little information has been available about these facts in regard to the screening for bacteriuria in non pregnant women Therefore the results of a *systematic 2 week nitrofurantoin therapy and a* 1 year follow up of female patients with bacteriuria found during the screening of a middle aged population (40–64 years) described in another article (34) are published below On the basis of these results the effectiveness of the therapy the need for further diagnostic examination and simultaneously the success of screening for bacteriuria in a middle aged female population were studied

SUBJECTS AND METHODS

Drug therapy and check-up of the results of treatment

Forty seven (85%) of the 55 female patients with bacteriuria found during the screening (34) were systematically treated by the physician conducting the project (J T) with a 2 week nitrofurantoin therapy (Nitrofur®-C Leiras 50 mg 3 times a day) A nitrofurantoin resistant bacterial strain was found in three of those who did not receive this therapy two had had to consult the attending physician because of acute UTI symptoms one was admitted to hospital and two were given antibiotics for some other disease One week after the therapy had been terminated a specimen of morning urine was checked with the dip-slide method (Uricult® Orion Diagnostica) Subjects whose urinary finding was less than 10^5 bacteria/ml were regarded as cured At the time of the check up a nurse asked about possible side-effects of nitrofurantoin

Follow-up

The subjects cured by nitrofurantoin therapy were followed up for 1 year or until recurrent urinary tract infection (UTI) was found in repeated dip-slide checks As in the study by Kunin (20) reappearance of significant bacteriuria was defined as recurrent bacteriuria The first

Table III *Bacteriurias recurring during a 1 year follow-up after nitrofurantoin treatment*

UTI treated earlier	Uncomplicated bacteriuria			Complicated bacteriuria			Total		
	Followed up (N)	Recurred N	Recurred %	Followed up (N)	Recurred N	Recurred %	Followed up (N)	Recurred N	Recurred %
Yes	15	10	67	5	3	60	20	13	65
No	13	9	69	1	1	100	14	10	71
Total	28	19	68	6	4	67	34	23	68

* Two patients are missing (one died one refused to participate)

UTI and 2 weeks is considered a suitable treatment period in among other things cases of asymptomatic bacteriuria (15) Generally speaking longer periods of medication have not been considered necessary the results of a 6 week treatment did not differ from those of a 2 week one (18) In our study the percentage of those cured was about the same as in some other studies (3 5) in which bacteriurias detected during screening of female populations were treated with short term (7–10 days) courses of nitrofurantoin

Diabetes (35) and hypertension (31) have been stated to predispose women toward UTI and these diseases must be considered as complicating factors when planning the treatment of UTI and in particular pyelonephritis (8 11 16) In our study rheumatoid arthritis was also considered to complicate treatment as only one of the four subjects with rheumatoid arthritis was cured of bacteriuria Although extensive kidney damage has been found in obductions of patients with rheumatoid arthritis (23) the number of UTIs in patients with rheumatoid arthritis has not been found to differ statistically significantly from that of controls (26) Since patients with rheumatoid arthritis generally use analgesics which have been found to damage the kidneys (25) their renal damage has been assumed to be due rather to the use of drugs than to UTI (26) It has also been suggested that rheumatoid arthritis causes renal damage regardless of analgesics (7) Since UTI has been found in the majority of patients with analgesic nephropathy assumed to have occurred only secondarily in already damaged kidneys (13) the poor result of treatment in our subjects with rheumatoid arthritis was possibly due to analgesic renal damage chronic UTI having attacked the kidneys secondarily

Previous UTI did not seem to have any negative effect on the results of the nitrofurantoin therapy However our results should be regarded with some caution because the information on previous UTI was based on the subject s recall only In fact we could not conclude with certainty the number of patients who had had an actual bacteriologically verified UTI because half of the general practitioners patients who complained of urinary discomfort did not have bacteriuria at the time of the examination (12)

Side-effects probably nitrofurantoin induced were found abundantly among our subjects For instance 11% of the women complained of nausea even though they had been asked to take the drug

Table IV *Results of a 1 year follow up after nitrofurantoin treatment for 14 days*

UTI treated earlier	Uncomplicated bacteriuria			Complicated bacteriuria			Total		
	Treated and followed up (N)	Cured no recurrence N	Cured no recurrence %	Treated and followed up (N)	Cured no recurrence N	Cured no recurrence %	Treated and followed up (N)	Cured no recurrence N	Cured no recurrence %
Yes	18	5	28	9	2	22	27	7	26
No	14	4	29	4	–	–	18	4	22
Total	32	9	28	13	2	15	45	11	24

Two patients are missing (one died one refused to participate)

with meals However when the subjects were questioned about side effects, no check list of possible symptoms was used because such a list has been found to elicit symptoms (14) Since according to Koch Weser et al (19), side effects caused by nitrofurantoin increase with the daily dose per patient weight and treatment time a comparison of the present side effects with those of other studies is not appropriate

During the 1 year follow up UTI recurred in about two-thirds of the subjects cured with nitrofurantoin Even though recovery among the uncomplicated cases was clearly better than among the complicated, no statistically significant differences were found in the proportional recurrences As in Mabeck s study (24) recurrences generally developed during the first 2 months following therapy The proportion of those cured of UTI with no recurrence within 1 year was less than half of that in the series of Asscher et al (3) The dissimilarity of the subjects in these two studies may account for the deviation for Asscher et al investigated 20–64 year old patients with no diabetes or UTI symptoms—in addition they had been screened from a selected female population

The benefit of screening for bacteriuria in non pregnant women is increasingly doubted, since [illegible] with bacteriuria that has been followed up [illegible] not developed chronic renal damage or [illegible] (2 9 32) The long term prognosis has also been good (4 27) for the majority of those who have had acute pyelonephritis It has been stated (28) that asymptomatic bacteriuria and UTI with recurrent symptoms hardly affect renal function even over a long period except in cases of simultaneous chronic poisoning (phenacetin) metabolic disease (diabetes) or obstruction of the urinary tract Confirmed conclusions of the harmlessness of asymptomatic bacteriuria can be drawn however only after a really long term follow up or follow ups of large numbers of subjects (6 17) Asscher et al (3) doubted the benefit of screening for bacteriuria when they realized that no therapy suitable for mass use was available for the bacteriurias found in their screening In their series the results of 1 week nitrofurantoin therapy did not in fact significantly differ from the placebo results obtained during a 1 year follow up It has already been stated that short term courses of antibiotics do not have any significant effect on long term results in UTI (10) Our results confirm earlier observations on the lack of therapeutic methods suited for mass use in cases of bacteriuria after screening Despite the 2 week nitrofurantoin treatment the majority of those cured had a recurrence of UTI already within the following year, regardless of the absence or presence of a disease that predisposed the patient to UTI or could have complicated the therapy

Several investigators (21, 22 33) have stressed the necessity of urography or even a complete urological examination of women whose first verified UTI is not cured or recurs Under these circumstances a total of 37 of our subjects (3% of all the women aged 40–64) would have needed further examinations (11 remained uncured UTI recurred in 23 and 3 already had a nitrofurantoin resistant bacterial strain as a result of recurring UTIs) If screening for bacteriuria were to include all middle aged women there would obviously not be resources for further examinations

According to our study, the benefit of screening for bacteriuria in middle aged women is questionable not only because of the lack of therapeutic methods suitable for mass use but also because of the inadequate facilities for further diagnostic examinations after screening and therapy

REFERENCES

1 Asscher A W Screening for disease Urinary-tract infection Lancet 2 1365 1974
2 Asscher A W Chick S Radford N Waters W E Sussman M Evans J S McLachlan M S F & Williams J E Natural history of asymptomatic bacteriuria (ASB) in non pregnant women In Urinary tract infection (ed W Brumfitt and A W Asscher) pp 51–61 Oxford University Press London 1973
3 Asscher A W Sussman M Waters W E Evans J A S Campbell H Evans K T & Williams J E Asymptomatic significant bacteriuria in the non pregnant woman II Response to treatment and follow-up Br Med J 1 804 1969
4 Bartels E D Pedersen K & Sardemann H Prognosen ved urinvejsinfektion hos kvinder Ugeskr Laeger 135 2814 1973
5 Brandberg Å Herner B Swartling P G & Vahlne G Urinvagsinfektion hos den vuxna befolkningen i en svensk stad Läkartidningen 67 2655 1970
6 Cattell W R The management of urinary-tract infection Practitioner 212 27 1974
7 Dieppe P A Doyle D V Burry H C & Tucker S M Renal disease in rheumatoid arthritis Br Med J 1 611 1976
8 Fass R J Klainer A S & Perkins R L. Urinary

tract infection Practical aspects of diagnosis and treatment JAMA 225 1509 1973

Freedman L R & Andriole V T A long term study of women with urinary tract infections In Abstracts 4th Int Congr Nephrology Stockholm vol 1 386 1969

Freedman L R Seki M & Phair J P The natural history and outcome of antibiotic treatment of urinary tract infections in women Yale J Biol Med 37 245 1965

Freeman R B Medical management of chronic pyelonephritis Acute and chronic Mod Treat 7 271 1970

Gallagher D J A Montgomerie J Z & North J D K Acute infections of the urinary tract and the urethral syndrome in general practice Br Med J 1 622 1965

3 Gault M H Rudwal T C Engles W D & Dossetor J B Syndrome associated with the abuse of analgesics Ann Intern Med 68 906 1968

4 Huskisson E C & Wojtulewski J A Measurement of side effects of drugs Br Med J 2 698 1974

5 Kasanen A Virtsatieinfektioiden kemoterapia Duodecim 89 1210 1973

— Kroonisen pyelonefriitin hoito Suom Laak L 28 1749 1973

7 Kass E H Should bacteriuria be treated? Med J Aust Special (Suppl) 1 38 1973

8 Kincaid Smith P & Fairley K F Controlled trial comparing effect of two and six weeks treatment in recurrent urinary tract infection Br Med J 2 145 1969

9 Koch Weser J Sidel V W Dexter M Parish C Finer D C & Kanarek P Adverse reactions to sulfisoxazole sulfamethoxazole and nitrofurantoin Manifestations and specific reaction rates during 2 118 courses of therapy Arch Intern Med 128 399 1971

0 Kunin C M Epidemiology and natural history of urinary tract infection in school age children Pediatr Clin North Am 18 509 1971

1 — Detection prevention and management of urinary tract infections Lea & Febiger Philadelphia 1974

2 Lang G R & Levin S Diagnosis and treatment of urinary tract infections Med Clin North Am 55 1439 1971

23 Lawson A A H & MacLean N Renal disease and drug therapy in rheumatoid arthritis Ann Rheum Dis 25 441 1966

24 Mabeck C E Treatment of uncomplicated urinary tract infection in non pregnant women Postgrad Med J 48 69 1972

25 Michielsen P de Schepper P J de Broe M Valkenborgh G & Tricot J P Renal diseases due to drugs In Drug induced diseases (ed L Mayler and H M Peck) vol 4 pp 261–324 Excerpta Medica, Amsterdam 1972

26 Mowat A G Hothersall T E & Gould J C Urinary tract infection in patients with rheumatoid arthritis Ann Rheum Dis 29 143 1970

27 Parker J & Kunin C Pyelonephritis in young women A 10- to 20-year follow up JAMA 224 585 1973

28 Ravnskov U Långtidsprognosen vid urinvagsinfektion och asymptomatisk bakteriuri Läkartidningen 72 1796 1975

29 Sallmander U Wallmark G & Ost C R Asymptomatisk bakteriuria hos hälsoundersökta kvinnor Läkartidningen 66 1669 1969

30 Sannerstedt R Lakartidningens behandlingsschema om hypertoni—en kommentar Lakartidningen 71 2170 1974

31 Shapiro A P Sapira J D & Scheib E T Development of bacteriuria in a hypertensive population A 7 year follow-up study Ann Intern Med 74 861 1971

32 Sourander L B & Kasanen A A 5 year follow-up of bacteriuria in the aged Gerontol Clin 14 274 1972

33 Straffon R A Urinary tract infection Problems in diagnosis and management 1973 Med Clin North Am 58 545 1974

34 Takala J Jousimies H & Sievers K Screening for and treatment of bacteriuria among a middle-aged female population I The prevalence of bacteriuria urinary tract infections under treatment and symptoms of urinary tract infections in the Sakyla Koylio project Acta Med Scand 202 69 1977

35 Vejlsgaard R Studies on urinary infection in diabetics I Bacteriuria in patients with diabetes mellitus and in control subjects Acta Med Scand 179 173 1966

36 Wilson J M G & Jungner G Principles and practice of screening for disease Public Health Pap no 34 WHO Geneva 1968

Acta Med Scand 202 81–85 1977

Renal Excretion of Pyruvate, Lactate and α-ketoglutarate in Kidney Donors before and after Nephrectomy and in Patients with Terminal Uremia

Poul Erik Skov and Hans Erik Hansen

From the First Medical University Clinic Århus Kommunehospital Århus Denmark

ABSTRACT The excretional patterns of lactate, pyruvate and α ketoglutarate have been investigated in 7 patients with terminal uremia and in 10 kidney donors with normal renal function before and after unilateral nephrectomy Methods for analysis of the three substances in urine were elaborated In all patients the levels of renal excretion of lactate and pyruvate were very low and clearance values were independent of the glomerular filtration rate (GFR) α ketoglutarate clearance varied to some extent with renal function but no correlation to GFR was found and exceeded the GFR in uremic patients indicating that the net result of renal handling of α ketoglutarate may be a tubular secretion

It is well known that lactate and pyruvate are subjected to rapid cellular metabolism only a small part of which takes place in the kidneys (8 11) 40% of α ketoglutarate is used by the liver 40% by the kidneys and only 20% is metabolized elsewhere primarily in the muscles Studies by Cohen et al (4 11) suggest that α ketoglutarate is taken up rapidly by the tubular cells through a mechanism which is similar to that which facilitates the uptake of para aminohippuric acid (PAH) whereas the excretion of α ketoglutarate to the tubular space involves a slow secretory process The net transtubular transport of α ketoglutarate is considered to be independent of the renal metabolism of α ketoglutarate (1 2 4 11 16) The transtubular transport mechanisms for lactic and pyruvic acid are unknown

The purpose of the present study was to investigate the renal excretions of lactate pyruvate and α ketoglutarate in kidney donors before and after unilateral nephrectomy and in patients with terminal uremia

MATERIAL

Ten living donors (four men and six women) were studied before and after unilateral nephrectomy All presented Hb BP serum electrophoresis and fasting blood sugar values within the normal range None had evidence of cardiac or pulmonary disease proteinuria or glucosuria Renal concentration capacity was normal based on a 26-hour urinary concentration test Other data are given in Table I Nephrectomy was carried out without complications

Seven patients 5 men and 2 women were uremic—as defined by a creatinine clearance of less than 10 ml/min All were clinically normohydrated and none received dialysis treatment All were anemic with hematocrits between 20 and 35% BP was increased in two patients but none had evidence of cardiac or pulmonary insufficiency Other data are given in Table I

METHODS

Study technique

The glomerular filtration rate (GFR) the effective renal plasma flow (ERPF) and the filtration fraction were determined in all patients Methods previously described were used in these investigations (13 14) When the studies were performed all patients had been fasting for 8 hours The renal clearances of lactate pyruvate and α ketoglutarate were determined concurrently with GFR and ERPF

Analytical methods

Lactate pyruvate and α ketoglutarate were measured using an enzymatic method (Boehringers BIO-chemica TEST Combination (3 9 10)) The serum concentrations of the three substances were determined by the above mentioned technique Serum containing α ketoglutarate in a concentration >4 8 mg/100 ml was diluted using redistilled water Zeiss and Beckman s (type DBG) spectrophotometers were used

The urinary content of lactate and pyruvate was determined by a modification of the method used for serum

Table I *Serum concentrations (mg/100 ml) of lactate pyruvate and α ketoglutarate in the patients studied*

	N	Serum lactate		Serum pyruvate		Serum α ketoglutarate	
		Mean	S D	Mean	S D	Mean	S D
Donors before nephrectomy	10	6 33	1 22	0 594	0 114	0 183	0 04
Donors after nephrectomy	10	6 43	1 53	0 448	0 144	0 236	0 05
Patients in terminal uremia	7	5 00	2 94	1 710	0 660	0 225	0 09

analysis Prior to determining the urinary content of lactate pyruvate and α ketoglutarate the following procedure was followed Freshly voided urine was prepared immediately with 0 6 perchloric acid and centrifuged at 3 000 r p m for 10 min

For lactate determination 300 μl of the supernatant were transferred to a test tube containing 2 00 ml sodium glycine hydrazine hydroxide buffer with a pH of 9 85 *Thereafter 0 03 ml LDH (Boehringer 2 mg LDH/ml) was* added followed by 0 20 ml NAD (Boehringer 27 mM) Specimens for determination of blind values were produced in the same way without addition of urine Specimens and blind values were placed in a water bath at 25 C for 1 hour and thereafter read at a pH of 7–8 in a Zeiss and Beckman spectrophotometer at 366 nm

For pyruvate determination 3 0 ml of the supernatant was added to 1 0 ml K_3PO_4 at a pH of 12 5 After 15 min in *an ice bath filtration was performed using Whatman no* 40 filter paper and the filtrate was warmed to 25 C in a water bath Afterwards the specimen was read at pH 11 5 against a urine blind at 366 nm After addition of 0 05 ml 0 012 molar NADH (Boehringer) E_I was read and after addition of 0 05 ml LDH (Boehringer 0 75 mg/ml) E_{II} was read 5 min later *The urine of uremic patients was diluted* 4 with redistilled water

For the determination of α ketoglutarate 3 0 ml of the supernatant *was added to 0 65 ml 1 0 mol* K_3PO_4 *after* which 3 9 ml 0 05 mol phosphate buffer at a pH of 7 4 was added The specimen was then cooled for 20 min in an ice bath After filtration through Whatman no 40 filter the filtrate was warmed to 25 C in a water bath and 3 9 ml of this solution at a pH of 7 1 was added to a cuvette A corresponding volume was added to a reference cuvette after which 0 05 ml 0 006 mol NADH (Boehringer) was added E_I was measured and 0 05 ml GLDH (Boehringer mg/ml) was added to the measuring cuvette and E_{II} was *read 5 min later Urine containing >5 mg/100 ml was* diluted with phosphate buffer Using redistilled water and urine from five normal persons and five uremic patients the reproduceability of the analysis was studied after addition of known amounts of the three substances together with dilution of the solutions containing known amounts of the three substances The recovery varied between 9? and 101% (Figs 1 2 3) Each series of investigations was followed by analysis of urine solutions containing known *amounts of each of the three substances mentioned* Recovery studies were performed within the ranges of 0 5–? mg/100 ml lactate 0 5–5 mg/100 ml α ketoglutarate and 0 3–0 8 mg/100 ml pyruvate in the urine All blood specimens were taken without compression

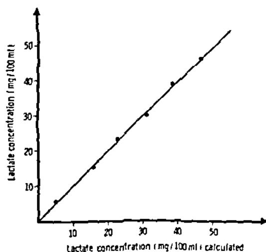


Fig 1 Recovery of lactate in urine from normals

RESULTS

The serum concentrations of lactate pyruvate and α ketoglutarate are given in Table I The lactate and α ketoglutarate concentrations varied only slightly within the three groups Lactate in the donors before and after nephrectomy was low but within the normal range and patients with terminal uremia showed values below the normal range α ketoglutarate concentrations were normal in all groups (17) Serum pyruvate in donors before and after nephrectomy was normal (17) while in patients with terminal uremia it was markedly increased

The clearance of both lactate and pyruvate in the patients studied was within the same range Clearance was completely independent of GFR and did not exceed 5 ml/min on an average in the three groups In patients with terminal uremia pyruvate clearance was almost identical with iothalamate clearance

In donors α ketoglutarate clearance was 9-25?

Table II *Kidney function studies in donors before and after nephrectomy*

C=clearance lact=lactate pyr=pyruvate α kg=α ketoglutarate Io=[125]iothalamate [131]I h=[131]I hippuran Excr=coefficient of excretion
All clearances are corrected to standard body surface (ml/min/1 73 m^2)

Donor no	Age (y)	Sex	C_{lact}	$Excr_{lact}$	C_p	$Excr_{pyr}$	$C_{\alpha\text{-}kg}$	$Excr_{\alpha\text{-}kg}$	C_{Io}	$C_{131_{I\ h}}$	M
Before nephrectomy											
78	50	♂	1 12	0 94	2 85	2 39	16 57	13 92	113	402	
79	42	♂	2 94	2 91	6 75	6 68	19 80	19 60	82	650	
81	44	♂	1 39	1 42	4 55	4 64	27 20	27 70	101	633	
88	52	♀	2 07	2 38	4 41	5 14	24 60	28 28	87	352	
89	53	♀	1 81	2 91	3 45	5 56	11 68	18 84	71	309	
91	19	♀	1 47	1 41	4 49	4 32	21 87	31 03	104	489	
94	44	♀	2 86	3 97	3 65	5 07	16 50	22 92	79	461	
95	40	♀	1 85	1 82	5 03	4 93	19 24	18 87	102	523	
109	65	♂	1 23	2 25	2 24	3 74	12 28	20 47	60	301	
111	56	♀	1 55	1 67	3 98	4 27	12 03	12 94	100	533	
Average	47		1 83	2 17	4 14	4 67	18 2	20 5	90	465	
S D			0 63	0 91	1 25	1 14	5 4	5 0	17	124	
S E M			0 20	0 29	0 39	0 36	1 7	1 6	5	39	
After nephrectomy											
78	53	♂	0 78	1 00	0 70	0 88	3 40	4 36	78	232	40
79	45	♂	3 28	3 38	2 28	2 35	10 44	10 76	97	342	40
81	47	♂	2 45	2 75	2 07	2 33	11 76	13 21	89	413	39
88	55	♀	2 57	3 73	1 58	2 29	17 09	24 77	69	212	35
89	56	♀	4 40	6 20	2 26	3 18	14 89	20 97	71	196	35
91	22	♀	3 47	3 69	2 61	2 74	26 11	27 78	94	318	35
94	47	♀	1 34	2 58	1 02	1 96	5 11	9 82	52	229	34
95	43	♀	3 01	3 46	1 72	1 98	12 06	13 86	87	310	32
109	67	♀	2 03	3 50	1 40	2 41	10 66	18 38	58	276	28
111	58	♀	1 20	1 41	1 50	1 76	14 32	16 85	85	283	26
Average	49		2 45	3 17	1 71	2 19	12 6	16 1	78	281	34
S D			1 34	1 43	0 60	0 61	6 3	7 2	15	67	
S E M			0 36	0 45	0 19	0 19	2 0	2 3	5	21	

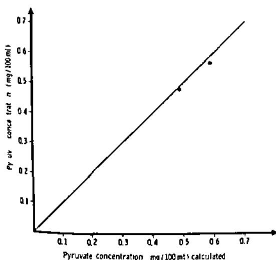


Fig 2 Recovery of pyruvate in urine from normals

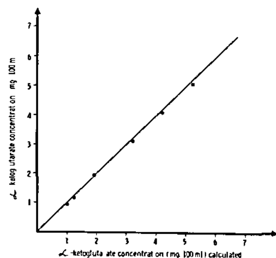


Fig 3 Recovery of α ketoglutarate in urine from normals

Table III *Kidney function studies in patients with uremia*
Abbreviations as in Table II

Pat no	Age (y)	Sex	C_{Io}	Hct	BP	C_{lact}	$Excr_{lact}$	C_{pyr}	$Excr_{pyr}$	$C_{\alpha kg}$	$Excr_{\alpha kg}$	C_{Hipp}
1	37	♂	8 80	31	175/120	1 31	14 88	7 76	88 18	7 71	87 61	37 90
2	47	♂	2 45	35	145/105	0 60	23 88	2 23	89 20	1 05	42 00	7 18
3	46	♂	1 88	20	120/80	1 00	52 63	0 16	8 31	2 79	146 89	5 27
4	46	♀	3 83	30	130/95	0 87	22 31	0 44	11 28	2 18	96 97	15 97
5	28	♀	4 94	25	135/90	1 06	21 20	2 46	49 20	7 36	147 20	21 70
6	17	♀	5 58	25	120/80	1 91	34 11	5 11	91 25	6 05	108 04	16 30
7	41	♀	3 65	22	140/75	1 05	28 37	4 45	120 27	2 37	64 04	20 00
Average	37		4 06	27	138/89	1 11	28 3	3 22	65 4	4 22	99 0	17 7
S D			2 63		19/20	0 41	12 3	2 71	43 2	2 97	39 4	10 7
S E M			0 99		7/8	0 16	4 6	1 03	16 3	1 04	14 9	4 3

of [125]iothalamate clearance (Table II) Both before and after nephrectomy a positive non significant correlation was found between the clearances of [125]iothalamate and α ketoglutarate as well as between the clearances of [131]I hippuran and α ketoglutarate (Table II) There was a significant difference in α ketoglutarate clearances before and after nephrectomy ($0\,1>p>0\,05$) The excretion coefficients before and after nephrectomy did not differ significantly α ketoglutarate clearance in patients with terminal uremia was identical to GFR (Table III)

DISCUSSION

[illegible] ies on the serum concentrations of lactate pyruvate and α ketoglutarate in normals revealed values within the same range as previously found (3 6 10 12) The principles for the analysis of the three substances in the urine were the same as those used for serum analysis but the techniques were modified so that the pH of the solution could be held within the specific range regardless of the pH of the urine The techniques employed could be used in all patients studied with a recovery percentage of 97–101 which corresponds to previously published results (12) Lactate in the serum in healthy donors was found to be low within the normal range In patients with terminal uremia average values were lower than the lowest normal values This is in accordance with the findings of Galloway and Morgan (5) whereas Guarnieri et al (6) and Tobin (15) reported increased lactate values in uremic patients

Serum pyruvate was increased in patients with terminal uremia as others have found (5 10) the explanation being a decrease in the production of acetyl CoA and $NADH_2$ in uremia It should be mentioned however that Guarnieri et al (6) found identical values for pyruvate in normal persons and uremic patients The α ketoglutarate concentration in the serum was identical in normals and in uremics in agreement with previously published results (6 17) The fact that α ketoglutarate concentration in the serum is the same in normals and in uremics can be explained by α ketoglutarate being metabolized in large quantities in the liver (4 7 11)

Lactate clearance was lower in patients with terminal uremia than in donors before and after nephrectomy In patients with terminal uremia higher average values were found for pyruvate clearance than in the donors and the excretion coefficient was 70% which corresponds to an elimination of pyruvate that is near the filtered amount The explanation for this is not clear and the finding is not in agreement with previous studies on patients with renal disease (17) reporting reduced clearances of α ketoglutarate and pyruvate

After unilateral nephrectomy α ketoglutarate clearance decreased The percentage clearance of this acid corresponded on an average to the reduction in renal function as measured by [125]iothalamate and [131]I hippuran clearances The excretion coefficient for α ketoglutarate was lower after unilateral nephrectomy but the difference was not significant

In uremic patients α ketoglutarate clearance was reduced still more but the amount excreted was identical to the amount filtered In two patients the clearance values were higher than the GFR as measured by [125]iothalamate clearance This suggests that α ketoglutarate is actively metabolized in

the kidney by a process which is identical to the mechanism whereby PAH is taken up in the tubular cells but where excretion from the tubular cells takes place according to an independent process (1 2 4)

REFERENCES

1 Balagura S & Stone W J Renal tubular secretion of alpha ketoglutarate in dog Am J Physiol 212 1319 1967
2 Baruch Balagura S & Stone W J Renal tubular secretion of p aminohippurate in the dog Effect of alpha ketoglutarate Nephron 6 633 1969
3 Bergmeyer H U Methods of enzymatic analysis Academic Press London 1965
4 Cohen J J & Wittmann E Renal utilization and excretion of alpha ketoglutarate in dog Effect of alkalosis Am J Physiol 204 795 1963
5 Galloway G E & Morgan J M Serum pyruvate and lactate in uremia Metabolism 13 818 1964
6 Guarnieri G F Bazzato G Bruschi E & Campanacci L L acetoacetato il 3 idrossibutirrato il lattato il piruvato e l d-chetoglutarato nell uremico cronico-sottoposto ad emodialiasi periodica G Clin Med 50 222 1969
7 Krebs H A & Cohen P P Metabolism of alpha ketoglutaric acid in animal tissues Biochem J 33 1895 1939
8 Landau B R Gluconeogenesis and pyruvate metabolism in rat kidney in vitro Endocrinology 67 744 1960
9 Lundholm L Mohme Lundholm E & Vamos N Lactic acid assay with L (+) lactic acid de hydrogenase from rabbit muscle Acta Physiol Scand 58 243 1963
10 Rosenberg J C & Rush B F An enzymatic spectrophotometric determination of pyruvic and lactic acid in blood Clin Chem 12 299 1966
11 Selleck B H & Cohen J J Specific localization of alpha ketoglutarate uptake to dog kidney and liver in vivo Am J Physiol 208 24 1965
12 Selleck B Cohen J J & Randall Jr H N Anal Biochem 7 178 1964
13 Skov P E & Hansen H E Glomerular filtration rate renal plasma flow and filtration fraction in living donors before and after nephrectomy Acta Med Scand 195 97 1974
14 — The functional pattern in patients with renal disease Acta Med Scand 196 387 1974
15 Tobin R B In vivo influences of hydrogen ions on lactate and pyruvate in blood Am J Physiol 208 24 1965
16 Yoshida T Yamashita F & Metcoff J Metabolic basis of aminonucleoside nephrosis in rats I Inhibition of alpha-oxyglutarate by aminonucleoside J Lab Clin Med 72 560 1960
17 Zelnicek E Changes of endogeneous alpha ketoglutaric and pyruvic acid in man Nature 185 928 1960

Acta Med Scand 202 87–88 1977

The Effect of Glucagon on Plasma Cyclic AMP and Glucose Concentrations in Patients with Alcoholic Cirrhosis

R C Strange O D Mjøs Thale Henden and P Jynge

From the Department of Clinical Chemistry University of Edinburgh Edinburgh United Kingdom and the Department of Physiology Institute of Medical Biology University of Tromsø Tromsø Norway

ABSTRACT Glucagon was infused intravenously into four patients with alcoholic cirrhosis and five healthy subjects and serial measurements were made of plasma cyclic AMP and glucose concentrations The results in the cirrhotic patients did not differ significantly from those in healthy subjects

Attempts to utilize diagnostically the association between liver disease and abnormal glucose metabolism (6) have proved disappointing For example Van Itallie and Bentley (5) were unable to differentiate patients with liver disease from healthy controls on the basis of differences in glucagon induced hyperglycaemia

Hepatic glucose production is regulated by the plasma concentrations of glucagon and insulin This control is exerted by hormone induced changes in the intracellular concentration of the nucleotide cyclic adenosine 3 5 monophosphate (cyclic AMP) (3 4) Although several tissues including liver can release the nucleotide into plasma in the basal state and after hormonal stimulation (3 4) it appears that the increased cyclic AMP concentrations found in plasma after i v administration of glucagon derive entirely from the liver (7)

The possibility that differences in plasma cyclic AMP concentrations after i v injections of glucagon can be used to differentiate patients with various types of liver disease from normal subjects has been examined with some success by Davies et al (1) We now describe the results of the test in four patients with well compensated alcoholic cirrhosis and in five matched healthy subjects

STUDY POPULATION AND METHODS

Four non hospitalized male subjects (age range 44–59 years weight range 71–110 kg) with well compensated alcoholic cirrhosis were studied In all cases the diagnosis was based on the pathology of liver biopsy specimens The control group (age range 49–63 years weight range 66–87 kg) comprised one female and four male in patients recuperating after minor orthopaedic surgery performed at least 10 days previously In all subjects studied the plasma concentrations of total bilirubin total protein albumin and thyroxine and the plasma activities of alanine aminotransferase and alkaline phosphatase were within reference ranges Informed consent for the procedures was obtained from all subjects

Patients were studied after an overnight fast After inserting a catheter into each antecubital vein two blood samples were taken with a 15 min interval from one vein and glucagon (100 ng/kg b wt /min) in NaCl (0 9% w/v) was then infused for 30 min into the other vein Blood samples were taken at 5 min intervals over 30 min and then at 10 min intervals over a further 30 min Blood was collected into heparinized tubes standing in ice immediately centrifuged and the plasma concentrations of cyclic AMP and glucose were measured Blood for cyclic AMP assay was collected in 10 ml tubes containing theophylline (50 mM) Cyclic AMP was measured with a protein binding method (The Radiochemical Centre Amersham Bucks UK) and glucose with a glucose oxidase method Statistical analysis was performed using the Wilcoxon test (2)

RESULTS

The mean plasma concentrations of glucose and cyclic AMP for both groups of subjects are shown in Fig 1 Although plasma glucose concentrations appeared to be higher in the cirrhotic patients at no

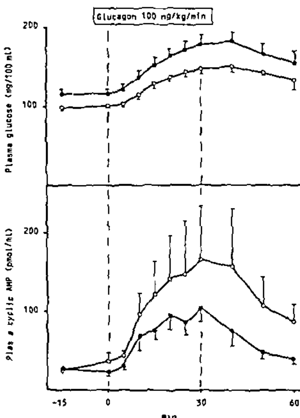


Fig 1 Effect of i v infusions of glucagon on the plasma concentrations of cyclic AMP and glucose in patients with cirrhosis (●) and in healthy subjects (○) Each point is the mean concentration ±1 S E M

time during the study were they significantly different from those of the control group Fasting plasma cyclic AMP concentrations were similar in both groups and although slightly higher they were not significantly different from values previously reported in similar healthy subjects (9) During the course of the glucagon infusion there was a considerable variation in cyclic AMP concentrations between individuals Thirty minutes after the start of the glucagon infusion the range of plasma cyclic AMP concentrations in the cirrhotic patients was 57–123 pmol/ml and in the healthy controls 34–342 pmol/ml Throughout the study there was no significant difference in cyclic AMP concentrations between the two groups

DISCUSSION

In agreement with the results of Davies et al (1) we found no significant difference in plasma cyclic AMP concentrations after administration of glucagon between healthy subjects and patients with well compensated alcoholic cirrhosis In contrast to our findings however Davies et al noted significantly higher fasting plasma cyclic AMP concentrations in patients with cirrhosis than in control subjects The reason for this difference is not clear although their control group was considerably younger (mean age 27 4 years) than ours

The failure to discriminate between healthy controls and patients with cirrhosis may partly be explained by the suggestion that diffusible cyclic AMP represents only a small percentage of the total hepatic content of the nucleotide even after enhancement of adenyl cyclase activity by glucagon (3) Measurement of cyclic AMP clearance from plasma does not appear to be a useful diagnostic aid either since although the liver can remove cyclic AMP from plasma (8) the clearance of cyclic AMP after cessation of the glucagon infusion was not significantly different in patients with cirrhosis compared with the normal subjects

REFERENCES

1 *Davies T F Prudhoe K & Douglas A P* Plasma cyclic adenosine 3 5 monophosphate response to glucagon in patients with liver disease Br Med J 1 931 1976
2 Documenta Geigy Scientific tables (ed Diem and Lentner) p 128 Geigy Basle 1970
3 Exton J H Lewis S B, Ho R J & Park C R The role of cyclic AMP in the control of hepatic glucose production by glucagon and insulin Adv Cycl Nucleotide Res 1 91 1972
4 *Exton J H Robison G A Sutherland E W &* Park C R Studies on the role of adenosine 3 5 monophosphate in the hepatic actions of glucagon and catecholamines J Biol Chem 246 6166 1971
5 *Van Itallie T B & Bentley W B A* Glucagon-induced hyperglycaemia as an index of liver function J Clin Invest 34 1730 1955
6 Megyesi C Samols E & Marks V Glucose tolerance and diabetes in chronic liver disease Lancet 2 1051 1967
7 Strange R C & Mjøs O D The sources of plasma cyclic AMP studies in the rat using isoprenaline nicotinic acid and glucagon Eur J Clin Invest 5 147 1975
8 Strange R C & Percy Robb I W Hepatic clearance of adenosine 3 5 cyclic monophosphate from plasma in the rat Biochem J 146 509 1975
9 Strange R C Rowe M J Mjøs O D & Oliver M F The effect of antilipolytic agents on cyclic AMP free fatty acid and total catecholamine concentrations in plasma Acta Med Scand 199 471 1976

Acta Med Scand 202 89–92 1977

Effects of Dexamethasone, Desoxycorticosterone, and ACTH on Serum Concentrations of Thyroxine, 3,5,3'-Triiodothyronine and 3,3',5'-Triiodothyronine

U Westgren B Ahren A Burger S Ingemansson and A Melander

From the Departments of Pharmacology Clinical Pharmacology and Surgery Lund University Hospital Lund Sweden and the Laboratory of Clinical Investigation University of Geneva Geneva Switzerland

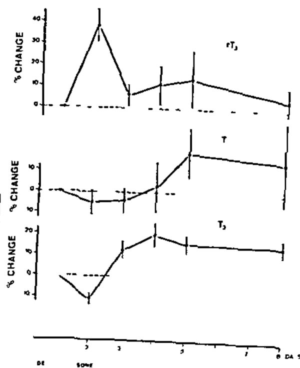
ABSTRACT The effects of a pure glucocorticoid dexamethasone and a pure mineralocorticoid desoxycorticosterone on the serum concentrations of thyroxine (T_4), 3 5,3 triiodothyronine (T_3) and 3,3 ,5 triiodothyronine (reverse T_3 rT_3) were compared both in healthy subjects and in athyreotic T_4-substituted patients In addition the effect of exogenous ACTH was examined in healthy subjects Both in healthy subjects and in T_4-substituted athyreotic patients administration of a single oral dose of dexamethasone caused a rapid and sharp decrease in the serum concentration of T_3 and a corresponding increase in the serum concentration of rT_3 The T_4 concentration was not changed A single oral dose of desoxycorticosterone evoked no significant changes in the serum concentrations of T_3, rT_3 or T_4 either in healthy subjects or in T_4 substituted athyreotic patients Like dexamethasone ACTH (two i v injections of 60 IU each at a 6-hour interval) evoked a serum T_3 reduction and a serum rT_3 increase Hence it appears that both endogenous and exogenous glucocorticoids but not mineralocorticoids may partially divert the deiodination of T_4 from the activating ($T_4 \rightarrow T_3$) to the inactivating ($T_4 \rightarrow rT_3$) pathway

The relationship between adrenocortical and thyroid function has been the subject of many studies Thyroid hormones are known to affect both the secretion and the metabolism of glucocorticoids (? 10) Glucocorticoids on the other hand influence thyroid hormone economy in various ways They diminish TSH secretion (13) and they affect the plasma protein binding of thyroxine (T_4) and 3,5 3 triiodothyronine (T_3) by reducing the binding capacity of thyroid hormone binding globulin (15) In addition it has recently been reported that administration of a potent synthetic glucocorticoid dexamethasone can evoke a diversion of T_4 monodeiodination from the activating (T_4 to T_3) pathway to the inactivating (T_4 to 3 3 5 triiodothyronine reverse T_3 rT_3) pathway (7)

While the effect of glucocorticoids on thyroid hormone levels has been studied by several investigators little is known about the possible influence of mineralocorticoids To explore this the present study was carried out It concerns a comparison of the effect of a pure mineralocorticoid 11-deoxycorticosterone (DOC) and that of a virtually pure glucocorticoid dexamethasone on the serum concentrations of T_4 T_3 and rT_3 In addition the effect of exogenous ACTH was examined The effects of dexamethasone and DOC were assessed both in normal subjects and in T_4 substituted athyreotic patients

STUDY POPULATION AND METHODS

The study was carried out on 20 euthyroid healthy volunteers and 9 athyreotic T_4 substituted patients All the patients were taking 0 25 mg T_4 (Levaxin* Nyegaard Oslo Norway) once daily The volunteers were divided into 4 groups of 5 who were given placebo tablets dexamethasone (Decadron* MSD Rahway N J USA) DOC (deoxycorticosterone acetate Doca* Organon Oss The Netherlands) and ACTH (Acton prolongatum* Ferring Malmö Sweden) respectively Of the 9 athyreotic patients 5 were given dexamethasone and 4 DOC Initial (control) venous blood samples were drawn before each treatment at 9 a.m on day 1 Dexamethasone and



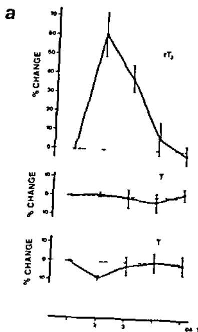
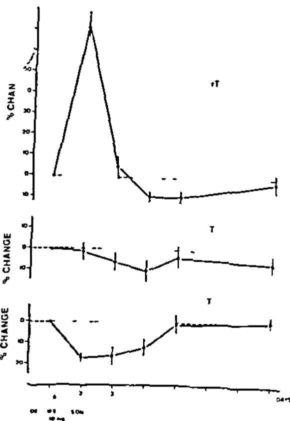


Fig 1 Percentage changes in serum concentrations of T_3, rT_3 and T_4 during administration of dexamethasone in healthy subjects (*a*) and in T_4 substituted athyreotic patients (*b*) and of corticotrophin (ACTH) in healthy subjects (*c*) Levels of statistical significance $^{*}p<0.05$ $^{**}p<0.01$ $^{***}p<0.001$



DOC were then given in single oral 10 mg doses and ACTH was given in a dose of 60 IU s c followed by another 60 IU 6 hours later Blood samples were then taken at 9 a m on the next five days The serum concentrations of T_4, T_3 and rT_3 were assessed by radioimmunoassays (3 5 12) Samples were analysed in triplicate (T_3 and rT_3) or duplicate (T_4) The results from each day were compared with those from day 1 by Student's *t* test

RESULTS

Dexamethasone (Figs 1*a* and *b*)

Administration of a single dose of dexamethasone evoked a rapid decrease in the serum T_3 concentration both in the healthy subjects and in the athyreotic patients In the latter the T_3 reduction occurred within one day and lasted for at least two days after which the concentrations gradually returned to initial levels In the healthy subjects the serum concentration of T_3 was also reduced within one day but returned to and exceeded the initial level within the second day

The serum concentration of rT_3 was increased within one day in both groups In the healthy subjects this increase was followed by a rapid normalization In the athyreotic patients the increase was

followed by a reduction to values below the initial levels

DOC

Neither in the healthy subjects nor in the athyreotic patients did administration of DOC evoke any significant changes in the serum concentration of T_4 T_3 or rT_3

ACTH (Fig 1*c*)

Within one day the two injections of ACTH evoked a reduction in the serum concentration of T_3 and an increase in that of rT_3 There was a return to initial levels within two days The serum concentration of T_4 did not change

Placebo

The serum concentrations of T_3 rT_3 and T_4 did not change during administration of placebo tablets

DISCUSSION

There is increasing evidence that the major production of the metabolically active T_3 and the metabolically inactive rT_3 occurs outside the thyroid by deiodination of T_4 (9 11) Recent observations suggest that this conversion of T_4 to T_3 and rT_3 is not a random process but is actively regulated Thus fasting and various illnesses seem to reduce the conversion of T_4 to T_3 and concurrently promote its conversion to rT_3 (4 6 14 16) In convalescence and after cessation of fasting there is a rapid normalization of the serum levels of T_3 and rT_3 Similar effects can be induced by administration and subsequent discontinuation of 6-propyl thiouracil (17)

A probable common denominator in fasting and disease is an increased adrenocortical activity (1 8) Accordingly an enhanced corticosteroid production might account for the diversion of T_4 deiodination from the activating (T_4 to T_3) to the inactivating (T_4 to rT_3) pathway in these conditions Indeed recent investigations have shown that short term administration of dexamethasone evokes a reduction and an increase respectively of the T_3 and rT_3 levels in serum of hyperthyroid patients as well as of T_4 substituted athyreotic subjects (7)

Also in the present study a single oral dose of dexamethasone promoted such alterations i e a decrease and an increase respectively of the serum T_3 and rT_3 levels In addition it was found that the effect was mimicked by injections of ACTH Hence it appears that not only exogenous but also endogenous corticosteroids can influence the T_3 and rT_3 serum levels Moreover as DOC failed to evoke such an effect it seems reasonable to assume that the corticosteroid influence on T_3 and rT_3 levels is associated with gluco- but not with mineralocorticoid action

As there was no significant change in the T_4 levels and as the T_3 and rT_3 alterations were recorded not only in normal subjects but also in athyreotic T_4 substituted patients the glucocorticoid effect was hardly the result of an influence on thyroid activity or on its hypothalamohypophyseal control system Furthermore even though corticosteroids are known to affect thyroid hormone binding by serum proteins (15) this is not a likely explanation of the present findings as a change in binding capacity would have influenced the T_4 levels at least as much as those of T_3 Accordingly the most probable interpretation in agreement with previous studies (7) is that both endogenous and exogenous glucocorticoids can partially divert T_4 deiodination from the activating pathway (T_4 to T_3) to the inactivating pathway (T_4 to rT_3) This conforms with the present finding that the effect of dexamethasone was more pronounced in T_4 substituted athyreotic subjects than in healthy volunteers as conversion of (exogenous) T_4 is the only way to generate T_3 and rT_3 in the former subjects If endogenous glucocorticoids participate in the regulation of T_4 conversion as assumed this could be one of the factors behind the T_3/rT_3 changes occurring in fasting subjects and during acute illnesses The mechanism whereby glucocorticoids act on T_4 deiodination remains unknown

ACKNOWLEDGEMENT

This investigation was supported by grant no 04X 3880 from the Swedish Medical Research Council

REFERENCES

1 Alleyne G A O & Young V H Adrenocortical function in children with severe protein-calorie malnutrition Clin Sci 33 189 1967

2 Brown H Englert E & Wallach S Metabolism of free and conjugated 17 hydroxycorticosteroids in subjects with thyroid disease J Clin Endocrinol 18 167 1958

3 Burger A Thyroxine radioimmunoassay In Clinical biochemistry Principles and methods (ed E Curtius and J Roth) 1st ed p 881 W de Gruyter Berlin 1974
4 Burger A Nicod P Suter P & Vallotton M B Reduced active thyroid hormone levels in acute illness Lancet 1 653 1976
5 Burger A Sakoloff C Staeheli V Vallotton M B & Ingbar S H Radioimmunoassays of 3 5 3 triiodo-L thyronine with and without a prior extraction step Acta Endocrinol (Kbh) 80 58 1975
6 Chopra I J Chopra V Smith S R Reza M & Solomon D H Reciprocal changes in serum concentrations of 3,3 ,5 triiodothyronine (reverse T_3) and 3,3 ,5 triiodothyronine (T_3) in systemic illnesses J Clin Endocrinol Metab 41 1034 1975
7 Chopra I J Williams D E Orgazzi J & Solomon D H Opposite effects of dexamethasone on serum concentrations of 3,3 ,5 triiodothyronine (reverse T_3) and 3,3 ,5 triiodothyronine (T_3) J Clin Endocrinol Metab 41 911 1975
8 Chopra M P Tahdani V Aber C P Portal R W & Parkes J Plasma cortisol urinary 17 hydroxycorticoids and urinary vanillyl mandelic acid after acute myocardial infarction Br Heart J 34 992 1972
9 Gavin L Castle J McMahon F Martin P Hammond M & Cavalieri R R Reverse T_3 and T_3 production rates in man Clin Res 24 143A 1976
10 Hellman L Bradlow H L Zumoff B & Gallager T F The influence of thyroid hormone on hydrocortisone production and metabolism J Clin Endocrinol 21 1231 1961
11 Larsen P R Triiodothyronine Review of recent studies of its physiology and pathophysiology in man Metabolism 21 1073 1972
12 Nicod P Burger A Staeheli V & Vallotton M B A radioimmunoassay for 3,3 ,5 triiodothyronine in unextracted serum method and clinical results J Clin Endocrinol Metab In press 1976
13 Nicoloff J T Fisher D A & Appleman M D The role of glucocorticoids in the regulation of thyroid function in man J Clin Invest 49 1922 1970
14 Nomura S Pittman C S Chambers J B Buck M W & Shimizu T Reduced peripheral conversion of thyroxine to triiodothyronine in patients with hepatic cirrhosis J Clin Invest 56 643 1975
15 Oppenheimer J H & Werner S C Effect of prednisone on thyroxine binding proteins J Clin Endocrinol 26 715 1966
16 Vagenakis A Burger A Portnay G I Rudolph M O Brian J T Azizi F Arky R A Nicod P Ingbar S H & Braverman L E Diversion of peripheral thyroxine metabolism from activating to inactivating pathways during complete fasting J Clin Endocrinol Metab 41 91 1975
17 Westgren U Melander A Wåhlin E & Lindgren J Divergent effects of 6-propylthiouracil on 3,5 3 triiodothyronine (T_3) and 3 3 ,5 triiodothyronine (rT_3) serum levels in man Acta Endocrinol (Kbh) In press 1977

Acta Med Scand 202 93–96 1977

The Clinical Value of Serum Triiodothyronine, Thyroxine and Thyrotropin Estimations during Medical Antithyroid Treatment

P Rogowski Th Friis C Kirkegaard and K Siersbæk Nielsen

From Medical Department E Frederiksberg Hospital Copenhagen Denmark

ABSTRACT The relation between clinical status and serial determinations of serum T_3 serum T_4 and serum TSH has been evaluated in the early phase of medical antithyroid treatment in 12 unselected hyperthyroid patients and in 19 patients who later during treatment accidentally developed low serum T_4 values Determination of both serum T_3 and serum T_4 was found necessary to avoid undertreatment Two patients with signs of hypothyroidism in the early phase developed low serum T_4 while serum T_3 and serum TSH remained normal In all of the 19 patients selected with low serum T_4 serum T_3 was normal Serum TSH was elevated in 5 patients without hypothyroid symptoms while 2 developed hypothyroid symptoms in spite of normal serum TSH values Our results suggest that serum T_4 is a more sensitive parameter than both serum TSH and serum T_3 in avoiding overtreatment during medical antithyroid treatment

The radioimmunological methods for the determination of serum triiodothyronine (T_3) used in clinical practice since 1970 have yielded additional information about the clinical significance of serum thyroxine (T_4) and T_3 in thyroid disease and the influence of both hormones on serum thyrotropin (TSH) levels (8) Serum T_3 determinations have been shown to be useful in the diagnosis of thyroid hyperfunction including T_3 toxicosis but of less value than serum T_4 and TSH in the diagnosis of hypothyroidism (3 6)

Data on serial estimations of serum T_3 T_4 and TSH during antithyroid treatment are few (1 5 13 15) Some of these studies included thyrotropin releasing hormone stimulation tests (TRH test) but this test has been found to lack clinical significance in the control of antithyroid drug therapy (10) Recent studies of thyroid hormone concentrations in patients who developed hypothyroidism early after strumectomy (14) and ^{131}I therapy (16) have shown transiently normal TSH levels with low serum T_3 and T_4 before clinical hypothyroidism These findings may suggest that serum TSH can be unreliable as a sensitive parameter also during medical antithyroid treatment

The purpose of the present study has been to evaluate the clinical significance of determinations of serum T_3 T_4 and TSH during medical antithyroid treatment

PATIENTS

The subjects of the study were 31 patients with hyperthyroidism The diagnosis was confirmed by the determination of serum thyroid hormone levels T_3 uptake test TRH test ^{131}I uptake ^{99m}Tc uptake and scintigraphy the clinical symptoms and effect of treatment

Twelve consecutive patients eight females and four males aged 28–85 years (mean 55) were followed before and during treatment at one week intervals for eight weeks Eight of the patients in this group had diffuse goiter and four multinodular goiter Another 19 patients 18 females and one male aged 30–82 years (mean 60) were selected from our Out Patient Department as persons who accidently later during antithyroid treatment had low serum T_4 values (low T_4 value) Diffuse goiter occurred in 16 and multinodular in 2 patients in this group while one patient had a solitary adenoma Two patients had exophthalmus This patient group was followed at up to three month intervals and data from the preceding visit were included (pre value)

Twenty-eight of the patients were given carbimazole two methylthiouracil and one propylthiouracil in conventional doses None of the patients received thyroid hormones or β blocking agents during the antithyroid treatment

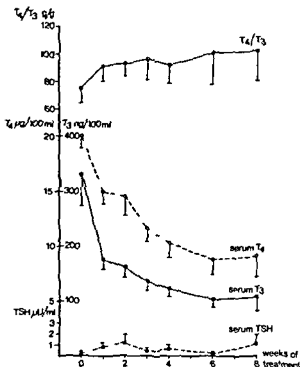


Fig 1 Serum TSH T_4 T_3 and T_4/T_3 ratio in 12 patients during the first 8 weeks of medical antithyroid treatment (mean±S E M)

METHODS

[S]erum T_4 was measured according to Murphy (11) (normal [ran]ge 5 0–11 0 µg/100 ml) T_3 uptake test using a commer[cial] kit (Triosorb Abbott) (normal range 0 80–1 20) Se[ru]m T_3 was measured by radioimmunoassay (6) (*normal* range (40–80 years) 38–126 ng/100 ml) and serum TSH by solid phase radioimmunoassay (10) (normal <4 9 µU/ml)) *Free T_4 index as an arbitrary measure of free T_4* was calculated as the product of serum T_4 and the T_3 uptake test (normal range 3 8–12 5) The ratio T_4/T_3 in serum was *calculated as g/g* and the mean value (±S D) in the age group 40–80 was 112±45 The statistical evaluation was made using Student s *t* test

RESULTS

*Fig 1 gives the results of serial simultaneous esti*mations of serum T_4 T_3 TSH and T_4/T_3 ratio in the group of 12 hyperthyroid patients before and during the first 8 weeks of medical antithyroid treatment The group in total showed a more rapid fall in serum T_3 than in serum T_4 in the first week but thereafter the majority of patients had parallel values of serum T_4 and T_3 The ratio T_4/T_3 showed a corresponding rise from a significantly decreased value to a constant and almost normal level In one of the patients in whom serum T_3 remained elevated at a time when serum T_4 had become normal during treatment, the clinical status was hyperthyroid corresponding to the high serum T_3 In three patients the opposite finding of normal serum T_3 with still elevated serum T_4 was noted only one of these patients was judged to be hyperthyroid Within the first eight weeks two patients developed low serum T_4 values with normal serum T_3 values Both became clinically hypothyroid for a short period but serum TSH remained normal None of the 12 patients developed high serum TSH within the first eight weeks

In the further follow up after eight weeks two additional patients showed low serum T_4 with normal serum T_3 In one patient serum TSH did not rise until low serum T_4 values had persisted for seven weeks In the other patient the decrease in serum T_4 was followed by an immediate rise in serum TSH The antithyroid treatment was adjusted and none of the patients developed clinical symptoms of hypothyroidism

The 19 patients who later during antithyroid treatment developed low serum T_4 values were divided into two subgroups one consisting of 5 patients (mean age 61 years) who developed high serum TSH and another of 14 patients (*mean age 60* years) in whom serum TSH did not rise Their serum TSH T_4 and T_3 values are given in Table I The thyroid parameters in the two subgroups were almost identical at the pre value examination Comparing the values at the time when serum T_4 had become low, mean serum T_4 was significantly lower in the subgroup with high serum TSH ($p<0\ 05$) An even more pronounced difference was found in free T_4 index (2 0±0 5 (mean±S D) and 3 2±0 9 respectively $p<0\ 005$) The mean serum T_3 *did not differ* and in both subgroups serum T_3 decreased to the same degree from the pre values The mean serum T_3 values in both subgroups were very close to the mean value in the control group of corresponding age (*40–80 years*) and none of the values were below the normal range The clinical status in the total group of 19 patients was evaluated but only two showed symptoms of hypothyroidism Neither of these two patients had high serum TSH at the time of evaluation

To evaluate a possible influence on the serum TSH response to low serum T_4 of the duration and severity of the hyperthyroid symptoms before treatment the period of clinical hyperthyroidism and the degree of serum T_4 elevation before treat

Table I. *Serum TSH, T_4 and T_3 in 19 patients developing low serum T_4 during medical antithyroid treatment (mean±S.D.)*

	Serum TSH (μU/ml)		Serum T_4 (μg/100 ml)		Serum T_3 (ng/100 ml)	
	Pre value	Low T_4 value	Pre value	Low T_4 value	Pre value	Low T_4 value
Patients developing high serum TSH (n=5)	2.3+2.2	17.8±7.9	6.9±2.2	2.6±0.9	100+35	84±27
Patients not developing high serum TSH (n=14)	2.0+2.5	1.9±1.3	7.0±1.8	3.6+0.8	97±33	78±18
Controls (n=90)	2.2±1.8		8.0±1.5		82±22	

* $p<0.05$ compared with patients not developing high serum TSH.

ment were estimated. No tendency to a longer duration or increased severity of symptoms was found in the subgroup without a rise in serum TSH. The mean duration of antithyroid treatment before the pre values was 2.5±2.7 months in those who did not develop high serum TSH, compared with 4.9±4.1 in those who did. This difference was, however, not statistically significant. The increase in serum TSH was also unrelated to the type of goiter and the occurrence of exophthalmus.

DISCUSSION

The present study was designed to assess the clinical value of serial determinations of serum T_3, T_4 and TSH in the early phase of medical antithyroid treatment. An attempt was also made to clarify the relation between the thyroid parameters and the clinical status in patients who later during antithyroid treatment developed low serum T_4 values. The early phase was characterized by considerable variations in the pattern of serum T_4 and T_3. Serum T_3 determination can be of value in avoiding under-treatment and we suggest that it should be undertaken whenever the clinical picture conflicts with serum T_4 values. Similar data have been described by Bellabarba et al. (1). In all the patients in whom serum T_4 values became low in the early phase, serum T_3 values were normal, but clinical symptoms of overtreatment developed in two despite the normal values of serum T_3 and TSH. In all patients serum TSH was normal within the first eight weeks and gave no information in relation to the clinical picture.

In agreement with our findings v. z. Muhlen et al. (13) recently described negative TRH test in spite of clinical hypothyroidism during medical antithyroid treatment. This pattern seems to correspond to the delayed response in serum TSH during the early phase following strumectomy (14) and radioiodine therapy in patients who consequently developed hypothyroidism (4, 16). A transiently impaired response of serum TSH to subnormal serum T_3 and T_4 values has also been described after withdrawal of exogenous thyroid hormone therapy in euthyroid patients (7, 17). In the majority of our patients, the antithyroid treatment was adjusted before manifest symptoms of overtreatment were noted but we find it likely that hypothyroidism would have developed later in most patients with persistently low serum T_4.

In four patients, clinical symptoms of hypothyroidism were noted for short periods with normal serum TSH both in the early phase (Fig. 1) and later during antithyroid treatment (Table I). In no patient did an elevation of serum TSH precede a decrease in serum T_4. Serum T_3 gave no information with regard to overtreatment and therefore serum T_4 seems to be the most sensitive parameter in avoiding it. The lack of an increase in serum TSH in patients with low serum T_4 could best be explained by the normal serum T_3 values, but this seems to be contradicted by our finding that some patients had high serum TSH and normal serum T_3 mean values, similar to the values of patients with normal serum TSH. Thus pituitary TSH release can be induced by low serum T_4 in some patients. This is in agreement with the assumption by Chopra et al. (2) that T_4 cannot be regarded as just a prohormone. In the patients with normal serum TSH, the lack of response in serum TSH to low serum T_4 might be explained by a slow reaction of the pituitary or production of immunologically abnormal TSH (18).

Factors which could possibly influence the relative unresponsiveness due to atrophy (12) or chemical damage of pituitary cells were evaluated but no relation was found between duration of symptoms or severity of hyperthyroidism and the response in serum TSH

Our results suggest that to avoid overtreatment, the most suitable parameter to follow is serum T_4 while serum TSH and serum T_3 are of less value as sensitive parameters of hypothyroidism during medical antithyroid treatment

ACKNOWLEDGEMENT

This study was supported by a grant from the National Institute of Arthritis and Metabolic Diseases Bethesda Maryland USA

REFERENCES

1 Bellabarba D Bénard B & Langlois M Clin Endocrinol 1 345 1972
2 Chopra I J Solomon D H & Teco G N C J Clin Endocrinol Metab 36 1050 1973
3 Evered D C Ormston B J Smith P A Hall R & Bird T Br Med J 1 657 1973
4 Gibberd M McMillan M & Staffurth J S Lancet 1 1048 1974
5 Golstein J Vanhaelst L Camus M & Glinoer D Horm Metab Res 8 128 1976
6 Kirkegaard C Friis T & Siersbæk Nielsen K Acta Endocrinol (Kbh) 77 71 1974
7 Krugman L G Hershman J M Chopra I J Levine G A Pekary A E Geffner D L & Teco G N C J Clin Endocrinol Metab 41 70 1975
8 Larsen P R Metabolism 21 1073 1972
9 Lauridsen U B Deckert T Friis T Kirkegaard C Hansen J M & Siersbæk Nielsen K Acta Med Scand 196 171 1974
10 Lauridsen U B Kirkegaard C Friis T & Siersbæk Nielsen K Acta Endocrinol (Kbh) 78 461 1975
11 Murphy B P J Lab Clin Med 66 161 1965
12 Murray S & Ezrin C J Clin Endocrinol Metab 26 287 1966
13 v z Muhlen A Hesch R D & Köbberling J Clin Endocrinol 4 165 1975
14 Sanchez Franco F Garcia M D Cacicedo L Martin Zurro A Escobar del Rey F & Morreale de Escobar G J Clin Endocrinol Metab 38 1098 1974
15 Shenkman L Mitsuma T & Hollander C S J Clin Invest 52 205 1973
16 Toft A D Irvine W J Hunter W M Ratcliffe J G & Seth J J Clin Endocrinol Metab 39 607 1974
17 Vagenakis A G Braverman L E Azizi F Portnay G I & Ingbar S H N Engl J Med 293 681 1975
18 Vanhaelst L Bonnyns M & Golstein-Golaire J J Clin Endocrinol Metab 41 115 1975

Acta Med Scand 202 97–101 1977

Mitochondrial Antibodies without Antinuclear Antibodies in Non-hepatic Diseases

Hans Diederichsen and Gunnar Pallisgaard

From Medical Department M and the Blood Bank Odense Hospital Odense Denmark

ABSTRACT The term "pseudo-LE syndrome" was previously used to describe an SLE like disease in which AMA, but not ANA were found in serum In an attempt to find patients with this syndrome we tested 9358 sera for AMA and ANA AMA without ANA was found in six patients without liver disease One of these patients had an SLE like disease Two of the others had diseases of the thyroid and one had rheumatoid arthritis—diseases in which AMA have previously been described One patient had allergic vasculitis and one hypercholesterolaemia these diseases are not known to be associated with the presence of AMA

The association between antimitochondrial antibodies (AMA) and primary biliary cirrhosis and chronic active hepatitis is well established (5 6 9 12) Furthermore AMA have been described in some autoimmune diseases such as rheumatoid arthritis Sjogren s syndrome and thyroiditis (11 13) Subclinical hepatitis was found in a number of patients with these diseases (11 13) AMA were demonstrated in some cases of systemic lupus erythematosus (SLE) (11) but not in others (6) Recently AMA were reported in patients with an SLE like disease the pseudo-LE syndrome (3 7 8) The absence of antinuclear antibodies (ANA) was taken to mean that these patients did not suffer from SLE (3 7)

The purpose of the present study was to examine whether an SLE like disease was present in patients with AMA but without ANA and without liver disease The study is based on sera received for routine testing for ANA

MATERIAL AND METHODS

The study is based on 9358 consecutive sera received for ANA testing and examined by means of the immunofluorescence method Ox liver was used as antigen The sera were tested for ANA and fluorescence corresponding to the cytoplasm of the hepatocytes A positive reaction with the cytoplasm of the hepatocytes may be caused by AMA (2 10) Sera reacting with the cytoplasm of the hepatocytes but not with cell nuclei were stored at −20°C for supplementary testing for AMA Only sera from patients without liver disease were included in the further study The patients from whom these sera were collected were examined for SLE according to the criteria of the American Rheumatism Association (ARA) (4) and for the SLE like disease as described by Maas et al (7 8) and Berg (3) Apart from those shared with the ARA criteria for SLE the cardinal findings in the pseudo-LE syndrome appear to be fever of unknown origin joint or muscle pains heavily increased ESR and leucocytosis (3 7) The patients were also examined for unrecognized clinical and biochemical signs of liver disease and liver biopsy was carried out if possible

Tests for AMA The stored sera from patients without liver disease which had presented hepatocytic cytoplasm reaction but no nuclear reaction were in addition tested for AMA against combined sections of fresh frozen liver tissue (2 10) kidney tissue (2 5 6 11) and stomach tissue (5 9 11) All tissues were obtained from ox The sera were considered to contain AMA if they reacted with the cells of the distal tubules and the ascending loops of Henle (10)

Immunofluorescence technique Sections of 6 μ were cut on a cryostat microtome dried in air incubated with inactivated serum diluted to 1 10 for 30 min at room temperature washed in phosphate buffered saline (PBS) pH 7 2 3 times for 5 min incubated with fluorescein conjugated (FITC) antihuman globulins for 30 min at room temperature washed in PBS 3 times for 5 min and covered with a mixture of equal volumes of PBS and glycerin and glass

Conjugates For the primary testing with liver tissue as antigen FITC antihuman globulin from the Baltimore Biological Laboratory was used during the first part of the test period and FITC antihuman IgG from the Behringwerke during the last part For the examination for AMA against combined sections of liver kidney and stomach FITC anti IgG and IgM from the Behringwerke were employed The same preparation was used throughout the study The FITC anti IgG had a molar F/P ratio of 2 3 The plateau

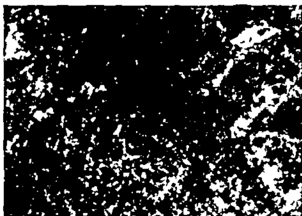


Fig 1 Immunofluorescent staining of granules in hepatocytes



Fig 3 Immunofluorescent staining of ascending loop of Henle

end point titre was 1 128 A dilution of 1 32 was used FITC anti IgM had a molar F/P ratio of 3 2 The plateau end point titre was 1 320 A dilution of 1 80 was used

Microscopic examinations were performed by means of a Zeiss fluorescence microscope with incident light interference filter KP 500 and barrier filter 50 The light source was an HBO 200 W mercury lamp

RESULTS

Immunofluorescence examinations

During the primary study of the 9358 sera 270 pre[sented] immunofluorescence staining of the hepato[cy]tes In all cases the fluorescence was coarsely granular scattered throughout the cytoplasm of the hepatocytes (Fig 1) The 270 sera were obtained from 69 patients Twelve of the patients who had no liver disease did not present ANA at this test (group A in Table I)

During a subsequent immunofluorescence study of the sera from these 12 patients against combined sections of liver kidney and stomach employing FITC anti IgG it was found that (*a*) apart from AMA one patient also had ANA in both sera according to this test (*b*) five patients had no AMA (negative reaction against kidney and stomach and during this test also negative reaction against hepatocytes) (*c*) five patients (nos 1 2 4 5 and 6) had AMA in all sera (positive reaction against distal tubules (Fig 2) and the loops of Henle (Fig 3) parietal cells (Fig 4) and hepatocytes) (*d*) one patient (no 3) had AMA (positive reaction against distal tubules and the loops of Henle negative reaction against parietal cells at this test also negative reaction against hepatocytes) Hence six patients with



Fig 2 Immunofluorescent staining of distal tubules

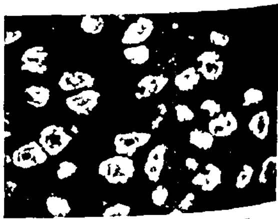


Fig 4 Immunofluorescent staining of parietal cells

Table I *Relationship to disease and ANA of sera giving granular fluorescence of hepatocytes*

Group	ANA	Liver disease	No of pats	No of positive sera
A	−	−	12	24
B	+	−	14	40
C	/	+	43	206

non hepatic disease (*c*) and (*d*) had IgG AMA without ANA in their sera One patient (no 2) had also IgM AMA

In five of the six patients without liver diseases and with AMA the maximum titre for IgG AMA against distal tubules ranged from 40 to 80 The lowest titre demonstrated was 1 20 In one patient (no 1) only one test was made shortly before her death In the other patients in whom two or more tests were made the reaction remained positive throughout the period of study which lasted 5–23 months

CASE REPORTS

Clinical examinations of the six patients with non hepatic diseases with AMA but without ANA

Patient 1 Woman born in 1895 From April to Aug 1974 she experienced poor appetite nausea vomiting loss of weight and pains in the epigastrium An examination could not explain these symptoms In Dec 1974 she was referred to a geriatric unit because of fatigue She was chronically ill with recurrent urinary infections In this connection there was a rise in leucocytes to 14 700/μl and increased ESR maximum 85 mm/h Clinical signs of an allergic vasculitis developed on the right lower leg and prednisolone was given with some effect In Feb 1975 she had an affection of the arm resembling herpes zoster During March 1975 she deteriorated and died presenting signs of a cerebral thrombosis No autopsy was performed

Examinations Hb 12 6 g/100 ml No liver biopsy One test for AMA in Jan 1975

Patient 2 Woman born in 1912 In 1958 a slight enlargement of the thyroid gland was observed Hashimoto goitre was diagnosed in 1965 by means of biopsy and since then she has been treated with L thyroxine

Examinations Hb 13 1 g/100 ml WBC 4 800/μl thrombocytes 416 000/μl and ESR 17 mm/h The thyroglobulin antibody titre 250 and complement fixing thyroid antibody titre (Wellcome) 8 AMA were demonstrated for the first time in Dec 1973

Patient 3 Woman born in 1930 One daughter suffers from discoid LE and has AMA and ANA Since 1953 the patient has had a false positive Wassermann reaction found for the first time during pregnancy In Jan 1974 she began getting black and blue spots of the skin after slight injuries and excessive menstruation On admission to a medical unit she had fever maximum 39 7°C and ESR of 126 mm/h Hb 5 5 g/100 ml thrombocyte count 5 000/μl Thrombocyte antibodies and leucocyte antibodies were found Prednisolone treatment was instituted Following this Hb went up to 12 4 g/100 ml and the thrombocyte count to 250 000/μl Coombs test was positive at repeated examinations

Other examinations the leucocyte count varied from 5 450 to 15 920/μl All differential counts were normal Urinary sediment contained tubular casts She refused liver biopsy AMA were found for the first time in April 1971

Patient 4 Woman born in 1912 In 1958 she developed rheumatoid arthritis and was treated with gold Since 1973 she has been under permanent treatment because of a gradual deterioration of her condition and has received i a prednisolone and naprosyne In November 1975 she was referred to hospital because of rheumatoid lung infiltration

Examinations Hb 12 5 g/100 ml WBC 6 500/μl thrombocytes 470 000/μl ESR 10 mm/h Liver biopsy revealed normal conditions AMA were found for the first time in April 1974

Patient 5 Woman born in 1905 Since 1930 she has had atypical joint pains In 1937 a non toxic struma was diagnosed She was admitted to hospital for the first time in 1962 for acromegaly In 1965 she received X ray treatment of the pituitary gland Since then she has had no signs of activity of the acromegaly In Oct 1974 she had an acute myocardial infarction In April 1975 she was referred to hospital because of fatigue and angina pectoris Because of the fatigue and the previous acromegaly her thyroid function was examined and found to be normal The thyroid gland could not be palpated Thyroid scanning revealed a cold node in the left lobe Hypercalcaemia was demonstrated Since then she has had intermittent moderate hypercalcaemia and is still being checked on an out patient basis for possible development of a parathyroid adenoma

Examinations Hb 13 9 g/100 ml WBC 8 000/μl thrombocytes 410 000/μl ESR 37 mm/h Serum calcium 2 82–2 77 mmol/l (normal range 2 29–2 67) serum phosphorus 1 22 mmol/l (normal range 0 79–1 59) Parathyroid hormone in serum 0 49 μg/ml (normal range 0 11–0 35) Growth hormone normal Thyroglobulin antibody titre 250 and complement fixing microsomal thyroid antibody titre (Wellcome) 16 AMA were found for the first time in April 1975

Patient 6 Woman born in 1928 Since 1950 she has suffered from attacks of migraine associated with paraesthesia of the left side of the face and the left leg In May 1974 she had neurodermatitis of the neck She has for several years had a tendency to xanthelasmata under the eyes but no xanthomas

Examinations Hb 15 0 g/100 ml WBC 8 800/μl thrombocytes 462 000/μl ESR 4 mm/h Serum cholesterol 10 5–9 4 mmol/l (normal range 3 2–8 2) serum triglyceride 1 69 mmol/l (normal range 0 44–1 85) She refused liver biopsy First test for AMA was made in May 1974

Table II. *Immunological investigations in patients with non hepatic diseases with AMA but wi*

Pat. no.	IgG (g/l)	IgA (g/l)	IgM (g/l)	C3 (μg/ml)	C4 (μg/ml)	RAT	LE cell test
1	9.5	1.63	0.79	/	/	–	–
2	13.4	1.66	0.46	/	/	–	/
3	8.1	0.70	2.75	695	20	–	–
4	8.2	1.58	0.58	995	350	+	–
5	7.2	1.16	0.16	1 390	400	–	–
6	9.8	2.04	0.36	Normal	225	–	–
Range	6.2–13.3	0.40–3.43	0.18–1.30	720–1 680	180–850		

The cardinal clinical diagnoses in these patients were: allergic vasculitis, chronic thyroiditis (Hashimoto), an SLE like disease, rheumatoid arthritis, polyglandular disease (adenoma of the pituitary gland and the thyroid gland and possibly parathyroid adenoma) and hypercholesterolaemia, in one patient each.

Criteria for SLE. One patient (no. 3) had some of the ARA criteria for SLE. She had a false positive Wassermann reaction, thrombocytopenia and Coombs positive autoimmune haemolytic anaemia and tubular casts in the urine. The remaining five patients presented none of the ARA criteria for SLE.

Findings characteristic of the pseudo LE syndrome (not included in the ARA criteria for SLE). Two patients (nos. 1 and 3) had leucocytosis. This ing cannot be taken into consideration in patient because she had a concomitant urinary infec one patient (no. 3) had fever and her ESR was increased to more than 80 mm/h, and one patient (no. 4) had pulmonary infiltrations. In the remaining patients, none of the clinical or laboratory changes described in the LE like disease were found.

Examinations for hepatic disease. None of these six patients had ever presented any symptoms of liver disease. Physical examinations revealed no enlargement of the liver or spleen or other clinical signs of liver disease. All had normal alkaline phosphatases, SGOT, bilirubin, prothrombin and thymol turbidity reactions.

Immunological tests. The results are shown in Table II.

DISCUSSION

For the purpose of detecting patients with the so-called pseudo-LE syndrome recently described in Germany (3, 7), we screened 9358 sera for AMA by means of the immunofluorescence method employing liver tissue as antigen. It is characteristic for patients with the pseudo-LE syndrome that they have AMA (not associated with liver disease) but not ANA (3, 7). Among the 9358 sera originally tested we found sera from six non hepatic patients which contained AMA without associated ANA. A decisive indicator that the sera from these six patients contained AMA was a positive reaction against distal tubules of the kidneys and against the ascending loops of Henle (10). None of these sera reacted exclusively against the proximal tubules without also reacting against the ascending loops of Henle as has been described for antibodies against the microsomal antigen (10). Sera with AMA react usually against parietal cells (5, 10). *Only in one of our six* patients did the sera not react against these cells. She was the only one with a disease resembling LE. She had three of the ARA criteria for SLE and also fever and a distinct ESR elevation (not caused by infectious diseases), which has been described as a cardinal finding in the pseudo-LE syndrome (3, 7).

The finding of only one patient with a disease resembling LE and with AMA among the more than 9000 sera tested could indicate that the syndrome described by Maas and Schubothe (7) is very rare. We might have found more sera with AMA if we had used kidney tissue as antigen in the primary investigation, because the distal tubules of the kidney give a more intense fluorescence reaction for AMA than do the liver cells (2). Furthermore, a higher F/P ratio of the conjugate (F/P 4.3) might have given rise to a higher number of positive reactions (1). One patient had a polyglandular disease comprising the thyroid gland, one had thyroiditis verified by biopsy, and one had rheumatoid arthritis, all of them diseases where AMA have been described previously (11–13).

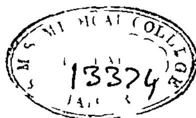
The implications of finding AMA in one patient with allergic vasculitis and in one with hypercholesterolaemia are not clear

ACKNOWLEDGEMENT

The investigation was supported by grants from Statens lægevidenskabelige forskningsråd and Fonden til lægevidenskabens fremme

REFERENCES

1 Andersen P & Beutner E H Defined immunofluorescence studies of cytoplasmic antibodies Int Arch Allergy 43 780 1972
2 Berg P A Mitochondrial antibodies in primary biliary cirrhosis I Localization of the antigen to mitochondrial membranes J Exp Med 126 277 1967
3 — Mitochondrial antibodies in non hepatic diseases Ger Med 3 58 1973
4 Cohen A S & Canoso J J Criteria for the classification of systemic lupus erythematosus status 1972 (Editorial) Arthritis Rheum 15 540 1972
5 Doniach D Roitt I M Walker J G & Sherlock S Tissue antibodies in primary biliary cirrhosis active chronic (lupoid) hepatitis cryptogenic cirrhosis and other liver diseases and their clinical implications Clin Exp Immunol 1 237 1966
6 Klatskin G & Kantor F S Mitochondrial antibody in primary biliary cirrhosis and other diseases Ann Intern Med 77 533 1972
7 Maas D & Schubothe H Ein Lupus erythematodes ahnliches Syndrom mit antimitochondrialen Antikorpern Dtsch Med Wochenschr 98 131 1973
8 Maas D Schubothe H & Droese M Das LE ahnliche Syndrom mit antimitochondrialen Antikorpern (Pseudo-LE Syndrom) Z Immunitaetsforsch 147 333 1974
9 Paronetto F Schaffner F & Popper H Antibodies to cytoplasmic antigens in primary biliary cirrhosis and chronic active hepatitis J Lab Clin Med 69 979 1967
10 Rizetto M Swana G & Doniach D Microsomal antibodies in active chronic hepatitis and other disorders Clin Exp Immunol 15 331 1973
11 Walker J G Doniach D & Doniach I Mitochondrial antibodies and subclinical liver disease Q J Med 39 31 1970
12 Walker J G Doniach D Roitt I M & Sherlock S Serological tests in diagnosis of primary biliary cirrhosis Lancet 1 827 1965
13 Whaley K Gondie R B Williamson J Nuki G Dick W C & Buchanan W W Liver disease in Sjogren s syndrome and rheumatoid arthritis Lancet 1 861 1970



Acta Med Scand 202 103-106 1977

Toxicity of Intravenous Catheters Tested in a Human Bone Marrow Culture System

B Thing Mortensen Lis E Jensen Søren Knudtzon and Nis I Nissen

From the Department of Medicine Finsen Institute and the Department of Pharmaceutics Royal Danish School of Pharmacy Copenhagen Denmark

ABSTRACT Nine brands of i v catheter were tested for toxicity in a human bone marrow culture system and content and leaching of plasticizers determined Three catheters showed toxicity to the culture system and three others leached di 2 ethylhexyl phthalate in appreciable amounts

Intensive medical treatment often requires daily i v administration of fluids and drugs for prolonged periods Infusion via needles or short catheters is technically simple but invariably leads to local vein problems due to the administration of irritant solutions such as potassium salts or cytotoxic agents Infusion via long indwelling catheters reaching the central veins reduces this type of toxicity and is now a routine procedure for example in the care of patients suffering from acute leukaemia or other generalized neoplastic disease

The main risk from these catheters is bacterial infection originating from the puncture site and it has been recommended to use a new catheter and a new site of introduction every 48 hours (2 8 16) It is impossible to follow this ideal method in clinical practice due to the lack of suitable veins and the usual clinical guideline is therefore to use a careful aseptic technique to inspect the puncture site and the vein every day and to remove the catheter at the first sign of infection

Even using these guidelines we have frequently observed thrombophlebitis of aseptic nature along the path of the catheter As the possibility existed that this was a chemical thrombophlebitis induced by the catheter itself we decided to examine whether the catheters in clinical use liberated substances toxic to human cells Due to our lack of culture systems for vascular endothelium we used in vitro growth of human bone marrow cells as test system

Reprint requests to B Thing Mortensen Department of Medicine Finsen Institute Strandboulevarden 49 DK 2100 Copenhagen Denmark

MATERIALS AND METHODS

Intravenous catheters were delivered in sterile packages and a new package was opened for each experiment All catheters were recommended for i v instillation except for no IX which is a baby feeding tube used as an i v catheter by our surgeons in the event of surgical access to the veins

The catheters were coded with numbers I Venocath® Abbott Laboratories Illinois USA II Bard I-Cath® C R Bard International Ltd Sunderland England III Intracath® Deseret Pharmaceutical Co Utah USA IV Subclavian Intrafusor® Sorenson Research Co Utah USA V E Z cath® Deseret Pharmaceutical Co Utah USA VI Trocaflex® Vygon Ecouen France VII Cavafix® B Braun Melsungen W Germany VIII Portex (i v cannula) Portex Ltd Kent England IX Argyle® (feeding tube) Sherwood medical industries Ltd Belgium According to the manufacturers catheters II and VIII were sterilized by radiation the others by ethylene oxide

The human cell culture system

A two-layer culture system was used with a bottom feeder layer of peripheral leucocytes (non-dividing) and a top layer of human bone marrow cells with dividing capacity and forming clusters and colonies of cells after a week of incubation (37°C) in humidified air with 7.5% CO_2 (14)

The feeder layer was prepared by mixing 10^6 washed peripheral leucocytes with 0 5% agar in McCoy s medium (Gibco) and 15% human serum 1 ml of this suspension was pipetted into each 35 mm Petri dish (A/S Nunc mark) before gelling occurred A 1 cm piece of th

Table I *Nine i v catheters tested in human bone marrow cell culture*

Catheter no	Material	No of experiments performed	Clusters and colonies (% of control) (Mean±S E)
I	PVC	5	87 2± 3 5
II	PVC	5	31 4± 8 2
III	PVC	5	26 2± 6 5
IV	PVC	2	82 0± 7 0
V	Teflon	3	96 0± 7 5
VI	Teflon	5	108 0±11 5
VII	Polyethylene	5	95 2± 6 3
VIII	Nylon	4	5 8± 5 8
IX	PVC	5	79 8± 6 4

ter to be examined was placed on top of the feeder layer in the middle of the plate The top layer mixture consisting of 2×10^5 normal human bone marrow cells/ml in 0 3% agar and 10% human serum was then added (1 ml per dish) to cover the catheter specimen Each experiment was done in triplicate cultures and with controls without a piece of catheter

After seven days of incubation the number of clusters (>5 cells) and colonies (>50 cells) was counted in a dissecting microscope at a magnification of×40 When inhibition was present cluster and colony numbers decreased in parallel and the pooled data are therefore used in Table I

Liberation of toxic substances was also tested by incubating catheter pieces in serum and in medium with 10% serum (serum medium) A 10 cm length of each eter (cut into pieces) was added to 6 ml of serum or m medium The catheter pieces were removed after ion (37°C) for 24 hours and 72 hours The serum and serum medium were then tested in cultures by the addition of 0 1 ml/dish and 0 2 ml/dish to the over layer before gelling occurred

Di 2-ethylhexyl phthalate (DEHP) is a commonly used plasticizer in polyvinyl chloride (PVC) tubing and the sensitivity of our culture system to DEHP was therefore of interest Pure DEHP (J J s (Chromatography) Ltd) was tested in various concentrations in serum 0 1 ml of the mixture being added to the upper layer containing the bone marrow cells

Determination of plastici ers

The PVC catheters were analysed for DEHP content and the amount of DEHP leached at 37°C from the catheters to serum and serum medium was measured In both cases DEHP was determined by gas chromatography using an internal standard for the quantitative determinations.

To determine DEHP in the PVC catheters solutions of the catheters in tetrahydrofuran (Merck) were used directly for gas chromatography (3 15) Together with the phthalates the analytical method used will also determine other commonly used plasticizers su h as adipates, citrates and sebacates

DEHP leached to serum has been described mainly in the lipoprotein fraction (12) DEHP was isolated from serum together with the lipids by extraction with a mixture of chloroform and methanol 2 1 (v/v) according to Folch et al (7)

The precision of the quantitative determinations of DEHP was estimated from ten single determinations and the relative standard deviation was found to be 1.5% in the analysis of the plastic tubing and 2 5% in the analysis of the serum extracts The limit of detection for DEHP was 0 6 mg/100 ml corresponding to 12 ng of the plasticizer All the results given below are expressed as the averages of two single determinations

RESULTS

The soft agar method of culturing human bone marrow cells showed sensitivity to toxic substances leached from some of the catheters tested Table I lists the results showing that catheters II III and VIII reduced cluster and colony formation significantly compared to controls For these 3 cathet rs the inhibition was about 70–95% Examination of the plates showed the remaining colonies to be

Table II *Leaching of DEHP to serum and serum medium after various times of incubation*
a and b refer to different serum samples

Catheter no	% w/w of DEHP		24 hours				72 hours			
			Serum		Serum medium		Serum		Serum medium	
			mg/100 ml	% w/w	mg/100 ml	% w/w*	mg/100 ml	% w/w*	mg/100 ml	% w/w*
I	24	a	9 4	3 6	2 7	1 0	23 2	8 8	4 0	1.5
		b	9 9	3 8	2 6	1 0	22 6	8 9	5.5	2 1
IV	13	a	3 7	2 3	1 5	0 9	4 9	3 0	2 5	1.5
		b	4 5	2 8	1 8	1 1	8 2	5 1	2 6	1 6
IX	23	b	9.5	0 6	3 4	0 2	23 8	1 4	5 5	0 3

* The tabulated figures indicate the amount of DEHP (% of the initial DEHP content) leached from the catheters

placed peripherally while the inhibition was total close to the incubated pieces of inhibitory catheters

This obvious liberation of toxic compounds from the inhibitory catheters was tested further by incubating pieces of each of the 9 catheters in serum and serum medium as described under Materials and Methods On addition of these solutions to the cultures catheter VIII gave nearly 100% inhibition in both experiments while no inhibition could be demonstrated with the other solutions

The inhibition demonstrated in the bone marrow cultures was in the first instance suspected of being due to the plasticizers especially DEHP and investigations were therefore undertaken to elucidate the content and release of plasticizers from PVC catheters No detectable plasticizer could be found in the inhibitory catheters II and III The non inhibitory PVC catheters such as I IV och IX contained and leached DEHP as shown in Table II The liberation of DEHP depends on incubation time catheter type and serum concentration Table II shows that during incubations for 72 hours up to 9% of the DEHP content is leachable and DEHP concentrations up to 24 mg/100 ml were found

The bone marrow cultures were not inhibited by these DEHP containing sera possibly because of the relatively low concentrations We therefore carried out experiments to determine the sensitivity of bone marrow cells to DEHP Pure DEHP was added to the culture in varying amounts without finding colony inhibition in concentrations up to 1 5 mg/ml

DISCUSSION

Toxicity has been found with reference to plastic materials in several culture systems often as the result of unexpected and secondary findings in experiments designed for other purposes In this way Duke and Vane (5) found that in a lung perfusion system the vascular response to hypoxia was blocked by inclusion of PVC tubing De Haan (4) demonstrated toxicity to heart cells isolated in tissue cultures when medium had been perfused through PVC tubing of the type used for blood transfusion Atkins et al (1) in 1968 demonstrated release of toxic factors from plastic components in a spleen perfusion system their test system being mouse bone marrow cultures The toxic factor leaching from the PVC tubing was not further identified Guess and Haberman (9) using several culture systems including human cells tested a variety of plasticizers and stabilizers They found toxic as well as non toxic compounds and concluded that it was technically possible to manufacture safe plastics for medical applications

The aim of our study was to find non toxic catheters for i v use Of 9 catheters tested 3 (including the 2 used in the department) were found toxic in our culture system Two of these toxic catheters were made of PVC but did not contain any plasticizers that we were able to determine The nature of the toxic compounds is therefore still unknown they might be plasticizers stabilizers or both

Three non toxic catheters were found to contain and leach DEHP to serum in appreciable amounts We were unable to demonstrate DEHP toxicity in our culture system even by the addition of high concentrations of pure DEHP to the cultures In contrast to our results with human bone marrow cultures DEHP has been shown to be toxic to other human in vitro cultures such as fibroblast cells (11) and diploid cell strain WI 38 (13) This toxicity was found in concentrations which could be achieved in blood stored in PVC blood bags for 21 days at 4 C It is therefore possible that clinical symptoms may appear in patients receiving DEHP from blood bags (12) from connecting tubing (6) and from i v catheters In fact clinical data have been presented which point to phthalate induced hepatic or enteric toxicity (10 17)

From any point of view it is desirable to avoid contamination by chemicals where possible and attention was paid to catheters V VI and VII which were neither toxic nor leached measurable amounts of chemicals However of these three catheters V and VI turned out to be clinically difficult to use due to frequent cracking at bends Catheter VII also has some technical disadvantages due to its double needle system and we can therefore not point to an ideal catheter for clinical use

As non toxic plastic and similar materials can be produced however and catheters V VI and VII are examples further efforts should be made in the pharmaceutical industry to develop devices which are both non toxic and technically perfect A number of biological test methods should be used to ensure non toxicity and among these the bone marrow culture system can be recommended for its sensitivity

ACKNOWLEDGEMENTS

The study was supported by the University of Copenhagen and by grants from the Danish Hospital Foundation for Medical Research and the Danish Medical Research Council

REFERENCES

1 Atkins R C Robinson W A & Eiseman B Cytotoxins released from plastic perfusion apparatus Lancet 2 1014 1968
2 Bolton-Carter J F Reduction in thrombophlebitis by limiting duration of intravenous infusions Lancet 2 20 1951
3 Burns D T Hayes W P & Steele P Identification and determination of primary plasticizers in polyvinyl chloride calendered formulations J Chromatogr 103 241 1975
4 De Haan R L Toxicity of tissue culture media exposed to polyvinyl chloride plastic Nature (New Biol) 231 85 1971
5 Duke H N & Vane J R An adverse effect of polyvinylchloride tubing used in extracorporeal circulation Lancet 2 21, 1968
6 Easterling R E Johnson E & Napier E A Plasma extraction of plasticizers from medical grade polyvinylchloride tubing (38389) Proc Soc Exp Biol Med 147 572 1974
7 Folch J Lees M & Stanley G H S A simple method for the isolation and purification of total lipids from animal tissues J Biol Chem 226 497 1957
8 Goldmann D A Maki D G Rhame F S Kaiser A B Tenney J H & Bennett J V Guidelines for infection control in intravenous therapy Ann Intern Med 79 848 1973
9 Guess W L & Haberman S Toxicity profiles of vinyl and polyolefinic plastics and their additives J Biomed Mater Res 2 313 1968
10 Hillman L S Goodwin S L & Sherman W R Identification and measurement of plasticizer in neonatal tissues after umbilical catheters and blood products N Engl J Med 292 381 1975
11 Jacobson M S Parkman R Button L N Jaeger R J & Kevy S V The toxicity of human serum stored in flexible polyvinylchloride containers on human fibroblast cell cultures An effect of di ?-ethyl hexyl phthalate Res Commun Chem Pathol Pharmacol 9 315 1974
12 Jaeger R J & Rubin R J Migration of a phthalate ester plasticizer from polyvinyl chloride blood bags into stored human blood and its localization in human tissues N Engl J Med 287 1114 1972
13 Jones A E Kahn R H Groves J T & Napier E A Phthalate ester toxicity in human cell cultures Toxicol Appl Pharmacol 31 283 1975
14 Knudtzon S Growth stimulation of normal human bone marrow cells in agar culture by human serum Scand J Haematol 12 298 1974
15 Lerche J & Mørch J Qualitative and quantitative gas chromatographic determination of plasticizers in polyvinyl chloride Arch Pharm Chem Sci Ed 1 25 1973
16 Maki D G Goldmann D A & Rhame F S Infection control in intravenous therapy Ann Intern Med 79 867 1973
17 Neergaard J Nielsen B Faurby V Christensen D H & Nielsen O F Plasticizers in P V C and the occurrence of hepatitis in a haemodialysis unit Scand J Urol Nephrol 5 141 1971

Acta Med scand 202 107–111 1977

Myocardial Scintigraphy with ^{99m}Technetium Stannous Pyrophosphate in Patients with Possible Acute Myocardial Infarction

K M Knutsen J E Otterstad and O Strøm

From the Departments of Clinical Chemistry and Internal Medicine Vestfold Central Hospital Tønsberg Norway

ABSTRACT Fifty six patients with a preliminary diagnosis of possible acute myocardial infarction (AMI) were studied on the second or third day after onset of symptoms by ^{99m}technetium stannous pyrophosphate myocardial imaging The scintigraphy was positive in 25 (44 6%) The final clinical diagnoses upon discharge were definite AMI in 11 with positive scintigraphy in 9 (82%), intermediate coronary syndrome (ICS) in 37 with positive scintigraphy in 15 (40 5%), postinfarction failure in 4 with positive scintigraphy in 1, no diagnosis of coronary heart disease in 4 patients with negative scintigraphy in all Of the 37 patients with a final diagnosis of ICS, 25 were admitted to the Coronary Care Unit with chest pain as the only symptom In this group the mean percentage increase in ASAT was significantly higher in 9 patients with positive scintigrams than in 16 with negative It is therefore assumed that among patients with ICS, a positive scintigraphy may reflect a more serious myocardial injury than a negative scintigram Of six patients with an acute tachyarrhythmia and ICS, scintigraphy was positive in the three with the most long lasting or severe arrhythmias False negative scintigrams may be seen in some patients with definite AMI

Myocardial imaging with ^{99m}technetium stannous pyrophosphate (^{99m}Tc PYP) is a sensitive method for detecting acute transmural and non transmural myocardial infarction provided the investigation is performed within 1–7 days after the onset of symptoms (8 9 10 14 15) Opinions differ however about the method s specificity Isotope uptake is demonstrated in some patients with unstable angina pectoris (1 8 10 15) with left ventricular aneurysms (2) cardiomyopathy (11) and in dogs after cardioversion (13)

The mechanism by which ^{99m}Tc PYP is taken up by infarcted myocardial cells is not completely understood Earlier studies suggested that the isotope uptake was mediated by an influx of calcium ions and pyrophosphate into mitochondria (6 9) Recent observations have failed to verify any quantitative relationship between calcium and pyrophosphate in infarcted cells (7)

The present study was carried out to investigate the value of ^{99m}Tc PYP in borderline cases i e patients clinically suspected of acute myocardial infarction (AMI) but in whom the conventional diagnostic methods were inconclusive at the time when scintigraphy was performed At the end of the hospital stay the diagnostic use of ^{99m}Tc PYP imaging was evaluated by comparing the scintigraphic results with the final clinical diagnosis

PATIENTS AND METHODS

Fifty six patients admitted to the Coronary Care Unit (CCU) during April–Sept 1976 were studied They all fulfilled our criteria for possible AMI on the second or third day after the onset of symptoms On admission they were grouped according to the main symptom (*a*) Acute chest pain (*b*) Acute left ventricular failure with concomitant chest pain (*c*) Tachyarrhythmia with concomitant chest pain Distributions by age sex and main symptom are given in Table I

Upon discharge the patients were divided into 4 groups according to the final diagnosis made independently of the result of the scintigraphy (*A*) Definite AMI (*B*) Intermediate coronary syndrome (ICS) (*C*) Postinfarction left ventricular failure (*D*) No coronary heart disease (CHD)

Table I. *Main clinical picture on admission, age range and sex distribution of 56 patients with possible AMI on the second or third day after onset of symptoms*

	No. of pats	Males	Females	Age (y.) Range	Mean
Chest pain	38	29	9	40–79	57.2
Acute left ventricular failure	12	9	3	52–82	69.4
Acute tachyarrhythmia	6	3	3	49–84	64.1
Total	56	41	15	40–84	64.2

Our criteria for definite and possible AMI were as follows:

Definite AMI. 1) *ECG abnormalities* involving development of new Q waves associated with changes in ST segments and T waves in specific and appropriate leads indicating the location of the infarct, or *both of* the following: 2) *Retrosternal chest pain* with or without radiation, duration ½ hour or more (typical chest pain). 3) *Aspartate aminotransferase* (ASAT) and *hydroxybutyrate-dehydrogenase* (HBDH) elevated and increasing (reference values ASAT 40 U/l, HBDH 350 U/l).

Possible AMI. Positive finding in *one* of the following: 1) *Typical chest pain* (see above), *or* sudden onset of *left ventricular failure* without known congenital or valvular heart disease, with concomitant chest pain. 2) *ECG abnormalities* compatible with transmural infarction. 3) *ASAT* and *HBDH* elevated. Positive finding in at least *two* of the following: 1) Atypical chest or epigastric pain resulting in admission to the CCU, or chest pain during tachyarrhythmias of sudden onset disappearing when sinus rhythm was restored. 2) ECG abnormalities with suspicion of coronary insufficiency (ST segment and T wave changes without development of new abnormal Q waves). 3) *ASAT* and *HBDH* borderline values.

Criteria applied for ICS in this study were: 1) Chest pain of more than 15 min duration. 2) ECG changes involving ST segment and T wave abnormalities with suspicion of coronary insufficiency. 3) ASAT and HBDH not exceeding the upper normal values.

No patient had a history of AMI in the six weeks prior to admission but 16 had suffered previous infarctions. None of the patients died during the hospital stay. All patients consented to participate in the study, and were subjected to continuous ECG monitoring during the scintigraphic procedure. Resuscitation equipment was available in the scintillation camera room.

^{99m}Tc PYP, 15 mCi (Isokit, Nyco) was injected intravenously. At 60–90 min after injection, myocardial scintigraphy (Nuclear Chicago Pho Dot IV scintillation camera) was performed in anterior, 60° left anterior oblique and left lateral projections as previously described (8, 9).

A low-energy, parallel hole collimator (15000 holes) was used, and images of 300000 counts were recorded. The scintigrams were graded independently by two of the authors. In the event of a discrepancy the scintigrams were reevaluated jointly. Localized activity of the radiotracer over the heart was taken as a positive scintigram. The extent or exact localization of uptake was not determined. The scintigrams were considered to be negative when no localized uptake was found or when doubt existed. False positive scintigrams attributable to uptake in breast masses or rib fractures were excluded clinically. None of the patients had been subjected to cardioversion before the scintigraphic examination. Coronary artery angiograms and left ventricular angiography were not performed.

Conventional statistical methods were used for calculation of mean values and S.E.M. The significance of differences between mean values was estimated by means of Student's *t* test.

RESULTS

The 56 patients suspected of having possible AMI at the time of the scintigraphic examination were divided into 4 groups according to the final diagnosis made upon discharge. In each group the result of scintigraphy was related to the main symptom on admission.

Table II. *Scintigraphic findings related to main symptom on admission in patients with final diagnoses of definite AMI (n=11) and intermediate coronary syndrome (ICS) (n=37)*

Main symptom on admission	No. of pats	Scintigraphic finding Positive	Negative
AMI			
Chest pain	10	8	2
Left ventricular failure	1	1	0
Total	11	9	2
ICS			
Chest pain	25	9	16
Left ventricular failure	6	3	3
Tachyarrhythmia	6	3	3
Total	37	15	22

Table III *Clinical data and scintigraphic findings in 6 patients with acute tachyarrhythmia with concomitant chest pain on admission and fulfilling our criteria for possible AMI on the second or third day after onset of symptoms*

AT=atrial tachycardia AF=atrial fibrillation SVT=supraventricular tachycardia SA=sinoatrial block VT=ventricular tachycardia N o =not observed Pa=paroxysmal ICS=intermediate coronary syndrome SSS=sick sinus syndrome

Pat no	Age (y)	*Sex*	Type of *arrhythmia*	Max *frequency*	Estimated *duration*	ECG after restored sinus *rhythm*	Final *diagnosis*	Scintigraphic *finding*
1	51	♀	AT	N o	5 h	Unspecific T wave changes	Pa AT ICS	Neg
2	79	♀	AF	220	26 h	Unspecific T wave changes	Pa AF ICS	Pos
3	84	♀	SVT SA	164	9 d	Unspecific ST T wave changes	SSS ICS	Pos
4	53	♂	VT	170	1½ h	Old posterior AMI ST T changes	Pa VT ICS	Pos
5	69	♂	AF	150	5 h	Unspecific ST T changes	Pa AF ICS	Neg
6	49	♂	SVT	N o	1½ h	Old posterior infarction ST T changes	Pa SVT ICS	Neg

Definite AMI

The final diagnosis disclosed definite AMI in 11 patients 10 presenting with chest pain and 1 with acute left ventricular failure Positive scintigrams were found in 8 patients with chest pain and in the patient with left ventricular failure (Table II)

Intermediate coronary syndrome

Thirty seven patients were discharged with this final diagnosis The main symptom was chest pain in 25 left ventricular failure with concomitant chest pain in 6 and tachyarrhythmia with chest pain in 6 Positive scintigrams were found in 15 (40 5%) (Table II)

There were 9 positive scintigrams among the 25 patients who had chest pain as the main symptom The mean (±S E M) percentage increase in ASAT was significantly higher ($p<0.05$) in these 9 patients (70±16) than in the remaining 16 with negative scintigrams (19±5) There was no significant difference in the mean percentage increase in HBDH between these groups (positive group 39±16 negative group 11±2)

Of the 6 patients admitted with acute left ventricular failure myocardial scintigraphy displayed a positive pattern in 3

Of the 6 patients admitted with a tachyarrhythmia with concomitant chest pain positive scintigrams were found in 3 Two of them had a long standing supraventricular tachyarrhythmia of 26 hours and 9 days duration respectively and the third had a ventricular tachycardia of 1½ hours duration The three patients with negative scintigrams all had supraventricular tachyarrhythmias of less than 5 hours duration (Table III)

Postinfarction failure

Four patients were discharged with this diagnosis All had had previous infarctions more than 6 weeks earlier and all were admitted with a left ventricular failure Routine clinical examination gave no evidence of recent infarctions but one patient had a positive scintigram His ECG was compatible with previous anterior and diaphragmatic infarctions and there was no increase in the enzyme values

No coronary heart disease

Four patients had no signs of CHD and scintigraphy was negative in all One of them was admitted with left ventricular failure and discharged with a diagnosis of hypertensive cardiac disease

DISCUSSION

In the present study we have demonstrated localized myocardial uptake in 9 of 25 patients admitted to the CCU with chest pain as the main symptom in 3 of 6 patients presenting with acute left ventricular failure and in 3 of 6 patients with an acute tachyarrhythmia all having a final diagnosis of ICS The overall prevalence of positive scintigraphy among patients with ICS was 40.5% According to our definition of ICS this group of patients is thought to have a spectrum of acute CHD between stable angina pectoris and definite AMI Abdulla et al (1) studied 10 patients with stable angina and found negative scintiscans in all Abnormal uptake was found in 5 out of 7 patients with unstable angina One patient showed a borderline picture Parkey et al (10) obtained positive scintigrams in 9 patients admitted with unstable angina pectoris In a previous study (8) we found one positive scintiscan in 10 patients with the diagnosis of unstable angina

It has been questioned whether ^{99m}Tc PYP myocardial imaging is a more sensitive technique for detecting small areas of myocardial necrosis than ECG and enzyme estimations or whether a positive scintiscan may also represent ischemic but not irreversibly damaged cells Zaret et al (16) demonstrated that ^{99m}Tc PYP uptake was maximal in the border zones of experimental infarction where only moderate CPK depletion had occurred They could not decide whether ischemic cells bind the tracer or if a positive scan images a population of damaged cells interspersed with normal cells in the border zone of infarction

Myocardial scintigraphy was positive in 9 patients presenting with chest pain and a final diagnosis of ICS The mean percentage increase in ASAT was significantly higher among these patients than in 16 patients with the same diagnosis but with negative scintigraphy As shown by Bergström and Säwe (3) patients with an ICS may have an enzyme pattern of AMI but with values within the normal range suggesting a minor infarction This might be reflected in the positive scintigrams in some of these patients

A positive scintiscan was observed in one patient discharged with postinfarction failure As he had suffered two infarctions previously the interpretation of his ECG might have obscured a new AMI X ray of the heart revealed no signs of left ventricular aneurysm but ventricular cineangiography would be necessary to exclude an aneurysm responsible for isotope uptake The mechanism of uptake by the presumably relatively avascular and fibrotic areas of the ventricle in left ventricular aneurysms is not understood (2)

In the patients with an acute tachyarrhythmia on admission two with a long lasting supraventricular arrhythmia and one with ventricular tachycardia and severe CHD had positive scintigrams. The three patients with negative scintiscans all had supraventricular arrhythmias of less than 5 hours duration This may indicate that in patients with CHD a long lasting tachyarrhythmia may lead to cellular injury or infarction not always detected by ECG or enzymes

We found 2 negative scintiscans among 11 patients with a definite AMI As outlined by Campeau et al (5) the incidence of false negative scintiscans may be higher than previously expected They found 2 (8%) false negatives among 26 patients with definite AMI Both our AMI patients with negative scintigrams had severe CHD with previous extensive anterior infarctions Both developed ECG evidence of new anterior extensive infarctions and ASAT and HBDH were elevated One of them had a clinically definite new AMI 10 days after admission We again obtained a negative scintiscan One might speculate whether earlier infarctions and widespread coronary artery disease account for this result The patient died later and the post mortem examination revealed severe coronary artery disease with total occlusion of the left anterior descending artery a recent anterior infarction and extensive scars after previous anterior infarctions A sufficient collateral circulation is thought to be essential for transport of the radiotracer to the freshly infarcted areas Several workers (4 12 16) have shown in animal experiments that blood flow has to be restored to deliver the radiopharmaceutical in which case the ^{99m}Tc PYP imaging is found to be an extremely sensitive technique for detection of AMI Infarctions as small as 1% of the left ventricular mass could be traced in a study by Bruno et al (4)

In conclusion ^{99m}Tc PYP myocardial imaging will give a positive pattern in about 40% of patients with ICS and in some patients with coronary artery disease and a long lasting or severe tachyarrhythmia. We have reasons to believe that among patients with ICS those whose scintiscan is posi-

tive may have a more severe CHD and should be observed and treated accordingly Further studies are needed to evaluate the short and long term prognosis in these patients Negative scintigraphy may be seen in some patients with a definite diagnosis of AMI

REFERENCES

1 Abdulla A M Canedo C I Cortez B C Mc Ginnis K D & Wilhelm W K Detection of unstable angina by 99mtechnetium pyrophosphate myocardial scintigraphy Chest 69 168 1976
2 Ahmad M Dubiel J P Verdon T A & Martin R H Technetium 99m stannous pyrophosphate myocardial imaging in patients with and without left ventricular aneurysm Circulation 53 833 1976
3 Bergstrom K & Sawe V Improved diagnosis of acute myocardial infarction by frequent serum enzyme determinations Acta Med Scand 193 515 1973
4 Bruno F P Cobb F R Rivas F & Goodrich J K Evaluation of 99mtechnetium stannous pyrophosphate as an imaging agent in acute myocardial infarction Circulation 54 71 1976
5 Campeau R J Gottlieb S Chandarlapaty S K C Mayorga-Cortes A & Miale A Accuracy of technetium 99m labelled phosphates for detection of acute myocardial infarction (Abstract) J Nucl Med 16 518 1975
6 D Agostino A N & Chiga M Mitochondrial mineralisation in human myocardium Am J Clin Pathol 53 820 1970
7 Dewanjee M K Kahn P C Dewanjee V & Connolly R J Mechanism of localization of Tc 99m labelled pyrophosphate and tetracycline in infarcted myocardium (Abstract) J Nucl Med 16 525 1975
8 Knutsen K M Otterstad J E & Strom O Hjertescintigrafi i infarktdiagnostikken (English summary) Tidsskr Nor Laegeforen 28 1472 1976
9 Parkey R W Bonte F J Meyer S L Atkins J M Curry G L Stokely E M & Willerson J T A new method for radionuclide imaging of acute myocardial infarction in humans Circulation 50 540 1974
10 Parkey R W Bonte F J Stokley E M Meyer S L & Willerson J T Analysis of Tc 99m stannous pyrophosphate (PYP) myocardial scintigrams in 242 patients (Abstract) J Nucl Med 16 556 1975
11 Perez L A Hayt D B & Freeman L M Localization of myocardial disorders other than infarction with 99mTc labelled phosphate agents J Nucl Med 17 241 1976
12 Poe N D Robinson G D & Tillisch J H Relation between blood flow and uptake of positive infarct imaging agents (Abstract) J Nucl Med 16 558 1975
13 Pugh B R Buja L M Parkey R W Poliner L D Stokley E M Bonte F J & Willerson J T Cardioversion and false positive technetium 99m stannous pyrophosphate myocardial scintigrams Circulation 54 399 1976
14 Willerson J T Parkey R W Bonte F J Meyer S L & Stokley E M Acute subendocardial infarction in patients Its detection by technetium 99m stannous pyrophosphate myocardial scintigrams Circulation 51 436 1975
15 Willerson J T Parkey R W Bonte F J Meyer S L Atkins J M & Stokley E M Technetium stannous pyrophosphate myocardial scintigrams in patients with chest pain of varying etiology Circulation 51 1041 1975
16 Zaret B L DiCola V C Donabedian R K Puri S Wolfson S Freedman G S & Cohen L S Dual radionuclide study of myocardial infarction Relationships between myocardial uptake of potassium-43 technetium 99m stannous pyrophosphate regional myocardial blood flow and creatine phosphokinase depletion Circulation 53 422 1976

Acta Med Scand 202 113–117 1977

Deposits of Plasma Proteins in the Skin during Treatment with Carbamazepine and Diphenylhydantoin

Henrik Permin and Leif Sestoft

From the Immunological Laboratory University Clinic for Infectious Diseases Rigshospitalet and the Departments of Neurology and Medicine F Gentofte Hospital Copenhagen Denmark

ABSTRACT Biopsies from skin of normal appearance from 18 patients treated with carbamazepine and diphenylhydantoin were investigated by a direct immunofluorescence technique Seventeen had deposits of plasma proteins at the dermoepidermal junction, 16 had deposits in the vessel walls, and one had autofluorescence of the nuclei in the epidermis and vessel walls These findings did not correlate with changes in serum IgG IgA, IgM, IgD, IgE or α_2-macroglobulin Eight patients had elevated alkaline phosphatase, 4 elevated IgG and one elevated IgA Three had low values of IgA, and all had normal values of IgM, IgD and IgE, and blood cells In three patients carbamazepine was withdrawn, whereupon the deposits disappeared in two and decreased in the third, who changed to another drug The changes were quantitatively and qualitatively similar to those seen in systemic lupus erythematosus induced by these drugs

Aplastic anaemia systemic lupus erythematosus (SLE) and erythema multiforme (15 28) are rare side-effects of carbamazepine and diphenylhydantoin (DPH) whereas skin rashes often with other manifestations of drug allergy have been reported to occur in about 3% of patients (7 28)

In patients with drug induced SLE and coeliac disease there have been reports of deposits at the dermoepidermal junction The deposits disappeared after withdrawal of the drug or after a gluten free diet (3 20 23)

The chance observation of autofluorescence of the nuclei of the epidermis and vessel walls in a patient who was being treated with carbamazepine led to a more thorough investigation of the effect of these drugs in the skin

PATIENTS AND METHODS

Skin biopsies and blood samples were taken simultaneously from 18 consecutive patients given carbamazepine (Tegretol®) and/or DPH (Difhydan®) Carbamazepine was given in a dose of 400–600 mg daily for one month to 4 years (mean 1 1/2 years) DPH was given in a dose of 200–300 mg daily for three months to 10 years (mean 4 years) The age and sex distribution and diagnosis of the patients are given in Table I None had collagenosis hypertension or signs of metabolic disease The serum anticonvulsant concentration was within the therapeutic range

Skin biopsies

Biopsy specimens from skin of normal appearance were taken from the forearm by a 4 mm punch technique following anaesthesia with ethyl chloride spray The tissue was immediately frozen and stored at −70°C until 4–6 μ thick sections were cut in a cryostat The sections were air-dried for 15 min washed in phosphate buffered saline pH 7 2 for 30 min and incubated with one drop of diluted conjugate in a moist chamber for 30 min Sections without any conjugate were controlled for autofluorescence and sections blocked with unconjugated antisera were included as controls

Antibasement membrane antibodies

Normal human skin and lip from a guinea pig were used as antigens The tissue was immediately frozen and stored at −70°C and investigated by indirect immunofluorescence technique as described earlier (22) The five immunoglobulins were used as conjugates and titers of sera ≥40 were considered abnormal

Antinuclear factors

Sera were investigated for the occurrence and titer of IgG and complement C3 fixing granulocyte specific antinuclear factors (GS ANF) and organ nonspecific antinuclear factors (ON ANF) (25 26 27) Rat liver cryostat sections and smears of isolated and washed human leucocytes served as nuclear substrates LE cell tests were studied by the method of Hammer (12) Rheumatoid

Table I *Clinical d ita (n tl e j atiei ts*

IF=id p thic ep lep y I F=postenceph I t c ep lepsy TI =p sttr u m tic ep lepsy TN=trigem n l neuralg a
A=IgA G=IgG M=IgM al=alb m n fib=fbrin gen Alk pl =alk l ne ph spl at se

I t no	Age (y)	Sex	D agn sis	Deps ts at the derm ep derm l junct n	Dep sits in the vessel walls
Carb m a ep ne					
1	51	♀	II	A it fl rescence (f tl e nucle	
2	45	♀	I I	M G κ λ C3 Fb	C κ λ Fb
3	37	♀	II	A C κ λ I	A M C κ λ al fb
4	45	♂	II	M G κ λ il C3	G κ λ al C3
5	45	♂	TI	M G κ λ al C3 Fb	A M G κ λ al C3 fb
6	5?	♂	TI	Fb	Fb
7	60	♂	TI	Fb	
8	61	♂	TN	A κ λ	A κ λ al Fb
9	5?	♂	TN	G κ λ Fb	G κ Fb
10	27	♂	II	I C3 Fb	M C λ al
D pl enyll ydant in					
11	23	♀	II	M G κ λ	C κ λ Fb
12	51	♀	TI	M G κ λ al	G κ λ al Fb
13	6?	♂	II	A G κ λ al C3	G κ λ il
14	41	♂	TI	G λ Fb	C κ λ C3 Fb
15	53	♂	II	M λ	il
16	43	♂	TI	G κ λ al Fb	G κ λ al
17	38	♂	II	G κ λ al Fb	G κ λ al Fb
Carbama ep e + I pl en ll l t in					
18	?7	♂	TI	M κ λ C3 Fb	κ λ al C3 Fb
N rm l values					

um or plasma concentration of

)	IgM (g/l)	IgG (g/l)	Alk ph (U/l)	α_2-macroglobulin (g/l)	
0	0 91	13 1	290	2 34	
9	0 26	15 2	240	2 25	
0	0 76	14 3	150	2 25	
3	0 51	7 9	305	2 46	
3	0 75	8 9	125	2 48	
0	0 34	12 5	160	1 68	
7	0 34	9 9	320	1 47	
1	0 61	11 6	180	1 23	
1	0 78	15 9	200	1 40	
5	0 82	9 9	140	2 05	
18	0 55	14 7	185	2 74	
75	0 69	16 4	290	2 42	
77	0 68	21 4	390	5 08	
61	0 39	14 3	180	2 46	
67	0 85	12 1	280	2 62	
11	0 65	14 3	495	1 67	
67	0 45	10 5	395	2 58	
77	0 34	14 3	190	2 25	
74–06	0 23–1 33	7 2–15 1	80–250	♀1 83–4 62	♂1 32–3 90

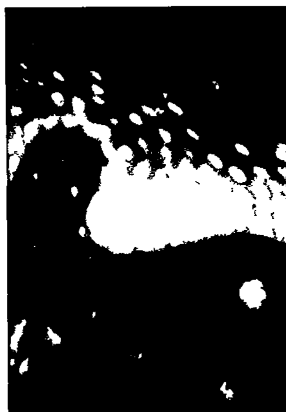


Fig 1 Autofluorescence of the nuclei in the epidermis

an anticonvulsant and in Jan the skin biopsy was repeated It now revealed no autofluorescence and no deposits in the skin ON ANF was unchanged as were the Ig concentrations No other drug had been given between April 1975 and Jan 1976

Case 2 A 45 year old woman suffering from epilepsy after an encephalitis in 1965 From Feb 1975 she had grand mal epilepsy and was given DPH but this was discontinued because of a rash From Dec 1975 she received carbamazepine in doses increasing from 200 to 600 mg daily but 3 weeks later she was readmitted with a rash A skin biopsy was performed one week after discontinuation of carbamazepine (Table I pat 2) The patient was given klonazepam and three months later a new skin biopsy showed only IgG at the dermoepidermal junction and vessel walls The serum Ig concentrations were unchanged

RESULTS

Deposits of plasma proteins were found in 17 of 18 skin biopsies from patients being treated with carbamazepine and/or DPH (Table I) Deposits of immunoglobulins were seen at the dermoepidermal junction in 15 patients Deposits of IgG were seen in 11 (Fig 3) IgM in 7 and IgA in 2 patients Fibrinogen was demonstrated in 9 albumin in 8 and complement C3 in 6 biopsies The deposits showed a granular and diffuse pattern All except one had deposits of a homogeneous or diffuse pattern in the vessel walls Thirteen had deposits of immunoglobulins The patient who had no deposits at the dermoepidermal zone had autofluorescence of the nuclei in the epidermis and vessel walls (Figs 1 and 2)

All patients with immunoglobulins also had κ and/or λ light chain deposition None had IgD IgE complement component Clq C3 activator or C9 or double stranded DNA deposits at the dermoepidermal junction or in the vessel walls

None of skin biopsies from 45 normal adults who received no drugs had deposits at the dermoepidermal junction Eight had homogeneous deposits in the vessel walls IgG in six albumin in 4 and fibrinogen in one

Carbamazepine and DPH showed the same pattern of deposition and no sex difference was fou



Fig 2 Autofluorescence of the nuclei in the vessel wall

None of the patients had circulating basement membrane antibodies using human skin and guinea lip as antigens

nly three patients had IgG ANF all ON ANF e in a titer of 1 16 and two in a titer of 1 256 one had complement fixing properties None had abnormal LE cell tests or rheumatoid factors Four patients had elevated serum IgG and one IgA Serum IgA was low in 3 patients and all had normal values of IgM (mean 0 59 g/l) IgD (mean 6 U/l) and IgE (mean 67 U/l) α_2 Macroglobulin was elevated in one patient and low in another and it did not correlate with the skin deposition The erythrocyte WBC platelet and reticulocyte counts and creatinine were normal Albumin (mean 4 8 mmol/l) LDH (mean 318 U/l) and SGOT (mean 27 U/l) were within the normal range in all patients while eight patients showed an elevated alkaline phosphatase (Table I)

DISCUSSION

Several mechanisms have been suggested to contribute to the deposition of plasma proteins at the dermoepidermal junction and vessel walls However, hypergammaglobulinaemia (3) antibodies against basement membrane (3 13 2?) or changes in α_2 macroglobulin (4) can be ruled out as can diabetes mellitus (17) in the present cases The deposits at the dermoepidermal junction found in our patients were similar to those seen in the skin of patients with systemic or drug induced lupus erythematosus with or without skin lesions (3 5 10 13 23) but our patients did not seem to have any symptoms or signs of lupus erythematosus Only 3 patients had IgG ON ANF and none GS ANF or complement fixing properties (2 9 23) while rheumatoid factors LE cell tests and blood cell counts were negative or within the normal range respectively

Administration of carbamazepine and DPH has been linked with a broader spectrum of immunopathies with impairment of both cellular and humoral responses (1, 9 11 15 21) Such a mechanism may serve to explain the levels of serum IgA in 3 patients and the elevated level of IgG in 4 The finding that 8 patients had slightly elevated values of alkaline phosphatase is in agreement with previous reports (16 19)

The deposits may be part of immune complexes The antigen component in such complexes seems to be the drug or one of its metabolites acting as hapten The antigen–antibody complexes circulate in the host and may react with plasma proteins or cells leading to injury of the tissues in which it is trapped (20) In the skin it may be the cause of a



Fig 3 Deposits of IgG at the dermoepidermal junction and vessel wall of the skin

ariety of injuries ranging from photosensitivity to rythema multiforme

On withdrawal of carbamazepine the deposits disappeared in one patient and decreased in another who changed to another drug Carbamaze pine can be rendered fluorescent (8) which can xplain the autofluorescence of the nuclei in the kin of one patient

The immunological changes in the skin previ ously reported to occur in SLE seem to be quantita tively and qualitatively of the same kind as those aused by carbamazepine and DPH in patients without symptoms or signs of lupus erythematosus The reaction may well be of more general im portance for the pathogenesis of side-effects of drug therapy

ACKNOWLEDGEMENT

This work was supported by the National Association against Rheumatic Diseases

REFERENCES

1 Aarli J A & Tonder O Effect of antiepileptic drugs on serum and salivary IgA Scand J Immunol 4 391 1975
2 Alarçon Segovia D & Fishbein E Patterns of anti nuclear antibodies and lupus activating drugs J Rheumatol 2 167 1975
3 Baart de la Faille Kuyper E H Lupus erythe matosus An immunohistochemical and clinical study of 485 patients Thesis Utrecht 1969
4 Brownlee M α_2 macroglobulin and reduced base ment membrane degradation in diabetes Lancet 1 779 1976
5 Burnham T K & Fine G The immunofluorescent band' test for lupus erythematosus III Employing clinically normal skin Arch Dermatol 103 24 1971
6 Committee on enzyme of the Scandinavian society for clinical chemistry and clinical physiology Recom mended methods for the determination of four en zymes in blood Scand J Clin Lab Invest 33 291 1974
7 Crill W E Carbamazepine Ann Intern Med 79 844 1973
8 Faber D B & Man in T Veld W A A thin layer chromatographic method for the determining car bamazepine in blood J Chromatogr 93 238 1974
9 Fontana A Grob P J Sauter R & Joller H IgA deficiency epilepsy and hydantoin medication Lancet 2 228 1976
10 Gilliam J N The significance of cutaneous immunoglobulin deposits in lupus erythematosus and NZB/NZW F_1 hybrid mice J Invest Dermatol 65 154 1975
11 Grob P J & Herold G E Immunological abnor malities and hydantoins Br Med J 2 561 1972
12 Hammer G A laboratory method for the description and evaluation of the LE phenomena in peripheral blood Scand J Clin Lab Invest 16 61 1964
13 Jablonska S Beutner E H Michel B Cormane R H Holubar K Bean S F Blaszczyk M Ploem J & Saikia N K Uses for immuno-fluorescence tests of skin and sera Arch Dermatol 111 371 1975
14 Johansson S G O Bennich H & Wide L A new class of immunoglobulin Immunology 14 265 1968
15 Kanoh T & Uchino H Immunodeficiency and epilepsy Lancet 1 860 1976
16 Killian J M & Fromm G H Carbamazepine in the treatment of neuralgia Arch Neurol 19 129 1968
17 Larsson O Studies of small vessels in patients with diabetes Acta Med Scand (Suppl) 480 1967
18 Laurell C B Quantitatively estimation of proteins by electrophoresis in agarose gel Anal Biochem 15 45 1966
19 Mosekilde L & Melsen F Anticonvulsant osteomalacia determined by quantitative analysis of bone changes Acta Med Scand 199 349 1976
20 Scott B B & Losowsky M S Coeliac disease a cause of various associated diseases? Lancet 2 956 1975
21 Sorrell T C Forbes I J Burness F R & Risch bieth R H C Depression of immunological func tion in patients treated with phenytoin sodium (sodium diphenylhydantoin) Lancet 2 1233 1971
22 Ullman S Halberg P & Nielsen R Anti basement membrane antibodies in sera from patients without bullous phemphigoid Acta Derm Venereol 55 305 1975
23 Ullman S Wiik A Kobayasi T & Halberg P Drug induced lupus erythematosus syndrom Acta Derm Venereol 54 387 1974
24 Weeke B Quantitatively estimation of human immunoglobulins following carbamylation by electro-phoresis in antibody-containing agarose Scand J Clin Lab Invest 22 107 1968
25 Wiik A Jensen E & Friis J Granulocyte specific antinuclear factors in synovial fluids and sera from patients with rheumatoid arthritis Ann Rheum Dis 33 515 1974
26 Wiik A & Munthe E Restrictions among heavy and light chain determinants of granulocyte specific antinuclear factors Immunology 23 53 1972
27 — Complement fixing granulocyte specific anti nuclear factors in neutropenic cases of rheumatoid arthritis Immunology 26 1127 1974
28 Zurcher K & Krebs A Hautnebenwirkungen in terner Arzneimittel III Teil einer Synopsis 4 und 5 Medikamente mit Wirkung auf autonom vegetative Funktionen und Organe Dermatologica 149 321 1974

Table I *Kinetic data for propoxyphene in plasma following intake of one Doleron tablet on an empty stomach and together with a standardized breakfast meal*

Subj no	C_{max} (ng× ml⁻¹)	t_{max} (min)	AUC×10³ (ng×min× ml⁻¹)
Fasting			
1	30	75	45 4
2	196	61	60 5
3	54	77	16 6
4	68	91	18 8
5	18	77	17 2
6	45	94	16 8
7	35	92	11 6
8	35	122	23 6
Non fasting			
1	82	53	28 2
2	130	130	79 1
3	69	88	22 7
4	114	61	24 6
5	41	63	11 7
6	62	90	22 5
7	37	91	12 1
8	29	237	13 4
Statistical significance of differences between fasting and non fasting conditions	N S	N S	N S

The tests were carried out as follows After total abstention from food and liquid for ten hours (10 p m -8 a m) the subject got a polyethene cannula inserted into an antebrachial vein and an initial blood sample (10 ml) was drawn (0-hour blank) Thereafter one Doleron tablet was ingested either with 100 ml drinking water or immediately after a standardized breakfast meal Doleron® (Astra Lakemedel Sodertalje Sweden) contains 65 mg D-propoxyphene chloride 350 mg acetyl salicylic acid 150 mg phenazone 50 mg caffeine and 5 mg Transergan® The breakfast was prepared by a dietician and consisted of 150 ml low fat milk 100 ml orange juice 1 egg 2 pieces of crisp bread 5 g margarine 20 g orange marmalade and 20 g cheese This equals 20 g (20%) protein 17 g (35%) fat 50 g (45%) carbohydrates and a total energy of 1 840 kJ (440 kcal) About 100 ml non sweetened black coffee was included The nurse collecting the blood samples surveilled eating and tablet intake When the tablet was taken on an empty stomach the subject abstained from food and liquid for another two hours after administration of the drug

Blood sampling

Blood samples (about 10 ml) were drawn before (0-hour) and at 15 30 45 60 75 90 120 240 360 480 min 24 and 48 hours after drug ingestion The exact time of blood sampling (when the sampling tube was half filled) was recorded and used in calculations and graphs

The blood samples were centrifuged and plasma was collected and frozen in three portions at -20°C until assessed for its content of propoxyphene norpropoxyphene acetyl salicylic acid plus salicylic acid and phenazone

Analytic methods

The plasma concentrations of propoxyphene and norpropoxyphene were measured by a mass fragmentographic method (1) The accuracy of the method was assessed by analysis of plasma samples containing varied known concentrations At a mean propoxyphene concentration of 23 ng/ml the relative S D of a single determination was 5% ($N=10$) and the mean deviation from the true value was 0 5 ng/ml At 186 ng/ml the corresponding values were 1% ($N=10$) and 0 03 ng/ml The detection limit was 4 ng/ml At a mean norpropoxyphene concentration of 1? ng/ml the relative S D of a single determination was 6% ($N=10$) with a mean deviation from the true value of 1 ? ng/ml At 122 ng/ml the values were 4% ($N=10$) and 0 03 ng/ml The detection limit was 3 ng/ml

The plasma concentrations of acetyl salicylic acid and salicylic acid were determined by a spectrofluorimetric technique (3) A linear relation was found in the concentration range 0–180 μg/ml The relative *S D* of a single determination was 4% at the 2 μg/ml level and the detection limit was 0 05 μg/ml

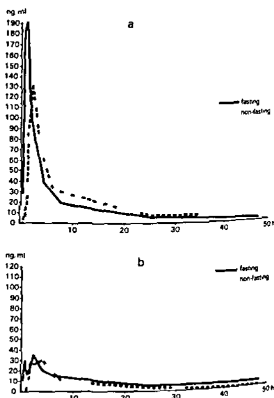


Fig 1 Plasma concentrations of propoxyphene in subjects 2 (*a*) and 8 (*b*) representing the highest and lowest observed concentrations respectively following ingestion of a single oral dose of Doleron

Table II *Kinetic data for norpropoxyphene in plasma following intake of one Doleron tablet on an empty stomach and together with a standardized breakfast meal*

Subj no	C_m (ng× ml⁻¹)	t_{max} (min)	AUC×10³ (ng×min× ml⁻¹)
Fasting			
1	55	244	72 8
2	103	91	108 0
3	65	92	105 6
4	78	91	80 2
5	50	249	44 7
6	49	94	74 3
7	48	239	67 5
8	41	477	61 9
Non fasting			
1	61	122	57 2
2	91	215	127 9
3	75	241	100 6
4	94	77	88 4
5	44	92	60 3
6	63	120	65 4
7	41	261	61 2
8	46	371	58 2
Statistical significance of differences between fasting and non fasting conditions	N S	N S	N S

The plasma concentrations of phenazone were determined by gas chromatography as recently described (4) This method is selective in relation to known metabolites and employs 4-methylphenazone as internal standard The relative S D of a single determination was 10 1% or 0 25 μg/ml in the concentration range 0 5–5 μg/ml

Calculations

The plasma concentrations of the respective compounds were plotted against time and the peak concentration (C_{max}) and time to peak concentration (t_{max}) were assessed and when relevant the elimination half lives were estimated The area under the plasma concentration curve (AUC) was calculated by the method of overlapping parabolas (2) including the infinite area The statistical significance of differences was calculated by paired t-tests

RESULTS

Propoxyphene

The peak concentrations of propoxyphene displayed a pronounced interindividual variation ranging from 18 to 196 ng/ml during fasting conditions and from 29 to 130 ng/ml in the postprandial state (Table I Fig 1*a* and *b*) The time to reach peak concentrations varied between 1 and 2 hours in the fasting state and between 1 and 4 hours postprandially (Table I Fig 1*a* and *b*) As the distribution of propoxyphene seemed to continue throughout the sampling period and no apparent elimination equilibrium was achieved no effort was made to assess elimination half life values

Like the peak concentrations the AUC values showed a considerable variation between individuals the difference between the extreme values being about 6-fold in the preprandial and about 7 fold in the postprandial state (Table I) The postprandial peak concentrations were higher than the corresponding preprandial values in five subjects whereas the opposite or no essential difference was observed in three (Table I) Totally the peak concentration values did not differ significantly between pre and postprandial conditions The postprandial AUC values were larger than the preprandial in four subjects whereas the opposite or no essential difference was seen in four (Table I) Totally the AUC values did not differ significantly between preprandial and postprandial conditions

Norpropoxyphene

Like propoxyphene norpropoxyphene (Table II Fig 2*a* and *b*) displayed a pronounced inter

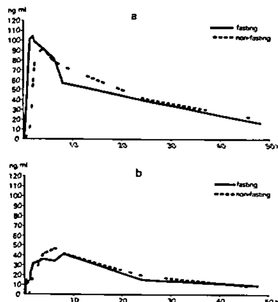


Fig 2 Plasma concentrations of norpropoxyphene in subjects 2(*a*) and 8(*b*) representing the highest and lowest observed concentrations respectively following ingestion of a single oral dose of Doleron

Table III *Kinetic data for acetyl salicylic acid plus salicylic acid in plasma following intake of one Doleron tablet on an empty stomach and together with a standardized breakfast meal*

Subj no	C_{max} (µg× ml⁻¹)	t_{max} (min)	AUC ×10³ (µg× min× ml⁻¹)	$t_{1/2}$ (h)
Fasting				
1	24 3	126	6 9	2 1
2	29 2	47	7 5	2 0
3	30 5	122	9 0	2 3
4	25 0	91	6 4	2 0
5	24 9	120	6 8	2 0
6	17 1	246	5 7	2 3
7	22 8	65	6 9	2 6
8	11 1	122	13 6	18 3
Non fasting				
1	30 2	53	6 8	2 1
2	19 0	130	6 9	2 8
3	20 5	241	8 4	2 9
4	26 7	61	6 3	2 0
5	28 1	45	5 9	1 8
6	17 1	240	6 5	2 9
7	18 9	113	6 7	2 1
8	9 6	237	6 8	6 6
Statistical significance of differences between fasting and fasting conditions	N S	N S	N S	N S

individual variation in peak concentrations (range 41–103 and 41–94 ng/ml during fasting and non fasting conditions respectively) and in time to peak concentrations (range 91–477 and 77–371 min during fasting and non fasting conditions respectively) The apparent elimination rate of norpropoxyphene was slower than that of propoxyphene (Fig 2*a* and *b*, Table II) and the AUC values showed only a 2 fold difference between individuals (Table II)

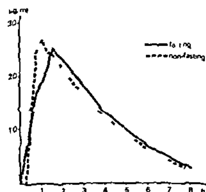


Fig 3 Plasma concentrations of acetyl salicylic acid plus salicylic acid in subject 4 following ingestion of a single oral dose of Doleron All subjects showed very similar curves

Acetyl salicylic acid and salicylic acid

The peak concentrations of acetyl salicylic acid plus salicylic acid varied between individuals The variation was less pronounced than that of propoxyphene (range 11 1–30 5 and 9 6–30 2 µg/ml during fasting and non fasting conditions respectively) (Table III Fig 3) The time to reach peak concentrations differed considerably from about 45 min to about 4 hours both in the pre and the post prandial state (Table III) The AUC values on the other hand showed very little variation both between individuals and between pre and postprandial conditions (Table III) With one exception also the elimination half life values ($t_{1/2}$) displayed very little variation between individuals (Table III)

Table IV *Kinetic data for phenazone in plasma following intake of one Doleron tablet on an empty stomach and together with a standardized breakfast meal*

Subj no	C_{max} (µg× ml⁻¹)	t_{max} (min)	AUC ×10³ (µg× min× ml⁻¹)	$t_{1/2}$ (h)
Fasting				
1	3 4	125	10 2	35 6
2	5 4	23	3 4	13 3
3	4 3	50	3 7	9 1
4	2 3	71	2 6	10 1
5	–	–	–	–
6	2 9	79	5 0	17 4
7	2 1	75	2 6	20 6
8	3 4	121	4 6	15 8
Non fasting				
1	5 5	37	10 0	31 7
2	3 1	130	3 7	14 3
3	3 2	119	3 2	11 4
4	3 0	30	2 3	11 1
5	–	–	–	–
6	3 1	119	2 9	10 3
7	2 7	29	4 0	21 4
8	2 7	29	5 5	17 1
Statistical significance of differences between fasting and non fasting conditions	N S	N S	N S	N S

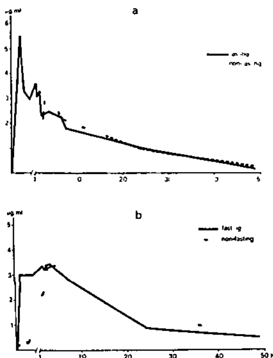


Fig 4 Plasma concentration of phenazone in subjects ?(*a*) and 8(*b*) representing the highest and lowest observed concentrations respectively following ingestion of a single oral dose of Doleron

Phenazone

Between individuals the phenazone peak concentrations showed a range of 2 1–5 4 (fasting) and 2 7–5 5 μg/ml (non fasting) The AUC values varied 4 fold between individuals during both fasting and non fasting (Table IV Fig 4) The time to reach peak concentration differed markedly from 23 to 125 min and from 29 to 130 min during pre and postprandial conditions respectively (Table IV Fig 4) Within individuals the pre and postprandial data differed but in a non systematic and non significant way (Table IV Fig 4)

DISCUSSION

The plasma concentrations of propoxyphene displayed a large variation between individuals In deed the peak concentrations and the AUC values showed a 10-fold and a 6-fold range respectively in the preprandial state Variations in the apparent volume of distribution may contribute to but not completely explain these large differences Hence it appears that the amount of propoxyphene from Doleron that reaches the systemic circulation is subject to a large interindividual variation This agrees with studies on other propoxyphene containing preparations (10)

It was recently reported that intake of various test meals either did not affect or slightly enhanced the bioavailability of propoxyphene given as propoxyphene chloride alone (10) In the present study a comparison of the intraindividual pre and postprandial AUC values for propoxyphene and its major metabolite norpropoxyphene revealed no systemic influence of food intake on the bioavailability of propoxyphene given as Doleron tablets Thus with respect to propoxyphene Doleron may be taken with meals as well as between meals

In addition to propoxyphene Doleron tablets contain two other analgesic components acetyl salicylic acid and phenazone As judged from the measurements of acetyl salicylic acid plus salicylic acid in plasma the absorption rate appeared to vary considerably between individuals On the other hand the elimination rate and the amount reaching the systemic circulation seemed to differ only slightly between individuals Moreover food intake did not systematically affect the bioavailability of acetyl salicylic acid from Doleron tablets As far as the third analgesic component phenazone is concerned the interindividual variation seemed to be considerable particularly with respect to absorption rate and amount absorbed Food intake on the other hand did not affect any kinetic parameter of phenazone in a systematic way

Thus it appears that food intake does not consistently influence the absorption rate or the bioavailability of any of the three analgesic components—D-propoxyphene acetyl salicylic acid and phenazone—that are contained in Doleron Hence this may be given together with meals as well as between meals However the large interindividual variations in propoxyphene and phenazone concentrations indicate that an optimal effect will not always be obtained by standard doses On the other hand observations derived from studies on single dose kinetics are not always applicable to multiple-dose conditions i e the usual therapeutic situation Therefore a study is being carried out in which the kinetics of the Doleron components are examined during t i d ake for a fortnight

REFERENCES

1 Bodin N O & Nygren R Determination of dextropropoxyphene and norpropoxyphene by a mass fragmentographic method To be published
2 Davis P J & Rabinowitz P Numerical integration Blaisdell Publishing Co Waltham Massachusetts 1967
3 Gustafsson B Agurell S Bodin N O & Örtengren B A study of possible interactions between substances in an analgesic combination product Doleron® Opusc Med To be published
4 Lindgren S Collste P Norlander B & Sjoqvist F Gas chromatographic assessment of the reproducibility of phenazone plasma half life in young healthy volunteers Eur J Clin Pharmacol 7 381 1974
5 MacDonald H Place V A Falk H & Darken M A Effect of food on absorption of sulfonamides in man Chemotherapia 12 282 1967
6 Melander A Danielson K Hanson A Jansson L Rerup C Scherstén B Thulin T & Wåhlin E Reduction of isoniazid bioavailability in normal men by concomitant intake of food Acta Med Scand 200 93 1976
7 Melander A Danielson K Hanson A Rudell B Scherstén B Thulin T & Wåhlin E Enhancement of hydralazine bioavailability to concomitant intake of food Clin Pharmacol Ther To be published
8 Melander A Danielson K Scherstén B Thulin T & Wåhlin E Enhancement of the bioavailability of spironolactone by concomitant intake of food Clin Pharmacol Ther To be published
9 Melander A Wåhlin E Danielson K & Rerup C On the influence of concomitant food intake on sulfonamide bioavailability Acta Med Scand 200 497 1976
10 Welling P G Lyons B S Tse M S & Craig W A Propoxyphene and norpropoxyphene Influence of diet and fluid on plasma levels Clin Pharmacol Ther 19 559 1976

Acta Med Scand 202 125–130 1977

Role of Bran in Normals

Serum Levels of Cholesterols Triglyceride Calcium and Total 3α-Hydroxycholanic Acid and Intestinal Transit Time

Jørgen Weinreich Oluf Pedersen and Kirsten Dinesen

From the Departments of Surgery I Medicine III Clinical Chemistry and Radiology Århus County Hospital Århus Denmark

ABSTRACT After the intake of approximately 24 g wheat bran daily for 5 weeks, 25 trainee nurses showed no changes in the serum levels of cholesterol, triglyceride calcium or total 3α hydroxycholanic acid On the other hand, the study revealed a reduced intestinal transit time with good correlation to an increased frequency of bowel movements Average body weight fell significantly, by 0 4 kg The daily caloric intake remained constant throughout the study period, whereas the calcium intake was significantly increased Among the serum parameters and the dietary constituents, good correlation was found only between serum cholesterol and the dietary cholesterol content In addition, an inverse relation ship was demonstrated between the serum levels of cholesterol and total 3α hydroxycholanic acid The significance of this observation is as yet unknown

Plant fibres mainly consist of lignin cellulose hemicellulose pectin and pentosans The content of these substances varies from plant to plant and with the age of the individual plants

In vitro experiments have shown that lignin can bind secondary bile acids such as desoxycholic acid (8) This binding of bile acids to plant fibres is therefore assumed to modify the action of bile acids in the intestine and to affect the faecal water con tent the fat excretion the influence of bile acids on the colon and the intestinal motility and transit time The binding of bile acid to plant fibres and the consequent increase in faecal excretion may exert an action on cholesterol metabolism (5) In one study (14) it has even been possible to demonstrate an influence on the serum levels of triglyceride and calcium

The laxative effect of plant fibres has been known for a long time (4) Epidemiological studies (2) on the intestinal transit time seem to show an inverse relationship between transit time and dietary fibre content

These problems have prompted several experi mental in vivo studies of the effect of plant fibres on serum lipids (3 6 18 22 25 26) The results of these studies are not consistent A possible expla nation for the discrepancies may be variations in the diet of the test subjects as such variations might influence the parameters measured (7 11) A gen eral feature of the studies has been the absence of dietary analyses

The study reported here was therefore performed under controlled conditions in order to find out whether wheat bran taken in physiological amounts (minimum dose 0 35 g/kg b wt /day) (17) had any effect on the serum levels of lipids calcium and total 3α hydroxycholanic acid and on the body weight intestinal transit time and the number of bowel movements per week For this use wheat bran has certain advantages over pharmaceutical preparations and other forms of plant fibre addition to the diet

STUDY POPULATION

The series studied consisted of 25 female (average age 21 years range 19–23) and 2 male trainee nurses (28 and 29 years) who had all their meals in the hospital canteen According to their own statements 16 subj had nor mal stools 1 loose 3 alternating hard a

Requests for reprints to J Weinreich MD University Clinic I of Surgery Århus Amtssygehus DK 8000 Århus C Denmark

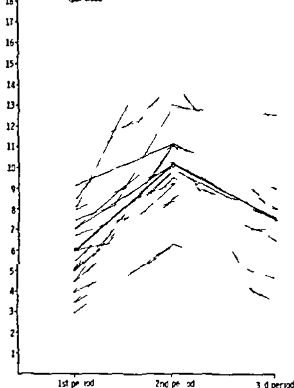


Fig 1 Number of bowel actions per week n 25 test subjects before during and after bran intake The heavy shows the average number

suffered from const pat on 4 of whom regularly used laxa t ves All the test subjects were in good health without subjective gastro ntest nal d scomforts other than those just mentioned The level of the r activity remained un changed throughout the study period Two subjects were excluded from the study one w th normal stool because of intercurrent disease and one w th const pation who ex perienced loose stools rrespect ve of the bran dose thus leav ng a total of 25 test subjects for analys s

METHOD

Before the start of the invest gation the tra nees were in structed to assess and record the r total da ly intake of food and flu d The invest gat on covered three periods of 2 5 and 2 weeks respect vely Record ng of the daily d etary intake was undertaken throughout the 9 weeks of the invest gat on In the second period an amount of 24 g coarsely ground wheat bran (part cle s ze ≤1 7 mm water absorpt on capacity 5 2 g/1 g bran) was added to the daily food The f rst and th rd periods served as control periods

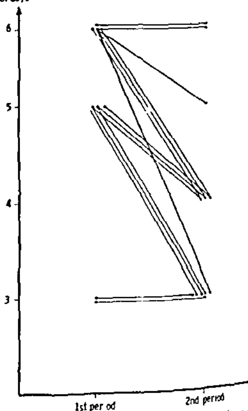
In the second week of the f rst control period and the fourth week of the test period da ly determ nat ons of the average food intake were made in each subject D etary analyses were performed with the ass stance of the Inst tute of Hygiene University of Århus by means of the international food tables incorporated in the computer programme (15) used by the Inst tute

Weekly determinat ons were made of the serum le el of cholesterol (normal range 4 0–8 0 mmol/l (27) triglyc eride (Boehringer K t for triglyceride (UV test) normal range 0 4–1 8 mmol/l) calcium (corrected to serum pro te n normal range 2 34–2 64 mmol/l (19)) and of the body we ght

The serum level of total 3α hydroxycholan c acid de termined by the method of Sch varz et al (29) as modif ed by Peterslund (28) (normal range 1 5–5 9 μmol/l) and the intest nal trans t time were determ ned at the end of the f rst and second period The trans t t me was measu ed by means of 200 ml Mixobar® suppl ed by Astra Hassle (0 6 g barium sulphate/ml) given in 4 dec ded doses o er one day The number of days taken for the contrast med um to traverse the intest ne was recorded and checked by daily fluoroscop c stud es Only 15 tra nees part c pated n the transit time determinat on because fluoroscopy should not be performed during the last fortn ght of the menstrual cycle The number of bowel movements per week was recorded

Stat st cal analys s

Wilcoxon s rank sum test for pa red data and Spearman s rho test with $p=0\,05$ as the l m t of s gn f cance were used



F g 2 Intest nal trans t t me before and during bran in take

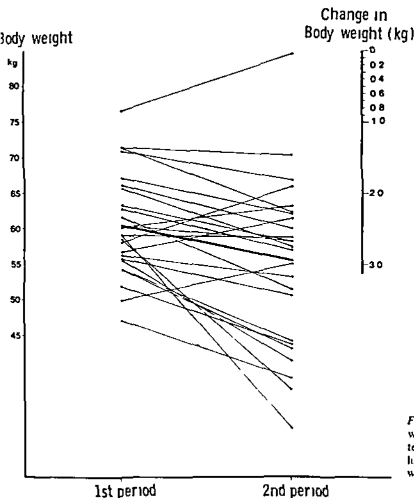


Fig 3 Changes in body weight in 25 test subjects after bran intake The heavy line shows the average weight loss

Table I *Serum levels of cholesterol triglyceride calcium and total 3α-hydroxycholanic acid in the three periods of investigation*

	1st period	2nd period	3rd period
Cholesterol (mmol/l)			
Median	5 00	5 08	5 06
Range	6 85–3 55	6 92–3 22	7 05–3 15
Triglyceride (mmol/l)			
Median	1 06	1 14	1 18
Range	1 50–0 60	1 62–0 68	1 90–0 55
Calcium (mmol/l)			
Median	2 50	2 48	2 47
Range	2 61–2 36	2 63–2 36	2 61–2 36
Total 3α hydroxycholanic acid (μmol/l)			
Median	3 6	3 6	
Range	5 8–2 2	5 8–1 6	

RESULTS

Among the test subjects with normal stools before the start of the investigation 4 had to reduce the bran dose to 12 g daily because otherwise the stools became too loose On the other hand in the trainees with loose stools the bran dose had to be increased to 36 g daily in order to obtain formed stools because 24 g daily resulted in mild constipation In the 3 trainees with alternating hard and formed stools the bran intake was followed by regular passages of naturally formed stools Of the 6 trainees with constipation 5—including the 4 who regularly used laxatives—had to increase the bran dose to 36 g in order to obtain regular bowel action The remaining trainee with constipation obtained a satisfactory effect on 24 g daily Thus none of these 6 required other forms of laxatives All the test

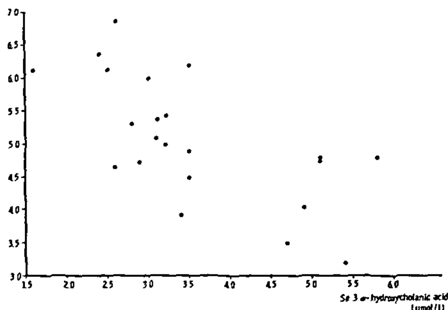


Fig 4 Serum cholesterol in relation to serum 3α hydroxycholanic acid (2nd period) Regression line $y=7.06-0.56\,x$ Spearman correlation coefficient $R=-0.65$ $p\leq 0.01$

subjects reported a subjective change in bowel movements with a looser easier and quicker defaecation during the period with bran intake and 13 intended to continue the bran intake after the experiment

Fig 1 shows the average number of bowel per week in the three periods The from 5 movements before to 10–7 during and after the bran intake is significant Fig 2 shows the change in the average transit time from 5 days in the first to 4 days in the second period This difference is also significant The transit time was inversely related to the number of bowel movements per week The average body weight was 60 4 kg at the start and fell to 60 0 kg at the end of the test period i e an average weight loss of 0 4 kg

Table II *Average daily consumption before and during wheat bran intake*

Figures within parentheses=1 S E

	1st period	2nd period
Protein (g)	64 1 (13 1)	64 3 (10 9)
Carbohydrate (g)	172 2 (31 8)	172 5 (37 7)
Total fat (g)	*76 1 (22 5)*	*76 2 (18 5)*
Saturated fatty acids (g)	33 4 (10 1)	34 1 (9 0)
Linolic acid (g)	8 2 (2 8)	7 7 (2 0)
Cholesterol (mg)	434 (118)	457 (100)
Sucrose (g)	62 4 (17 6)	56 6 (17 0)
Calcium (mg)	905 (239)	1 037 (222)

which cannot be regarded as fortuitous occurred within 5 weeks (Fig 3)

Table I shows the median values and ranges of the serum levels of cholesterol triglyceride calcium and total 3α hydroxycholanic acid in the three periods No significant changes occurred during the period of bran intake A correlation analysis revealed an inverse relationship between the serum levels of cholesterol and 3α hydroxycholanic acid in both of the two periods in which measurements were performed (Fig 4)

The results of the dietary analysis are shown in Table II We found a significant increase in the calcium intake The other dietary constituents listed showed no significant changes The analyses revealed a direct relationship between the dietary cholesterol content and serum cholesterol in the two periods studied but there was no other form of correlation between the other dietary constituents and the serum parameters

DISCUSSION

The reduction observed in the intestinal transit time after an increase in dietary fibre is in good agreement with the findings by others (13 26) However a few authors have been unable to demonstrate such a change The explanation for this might for example be a too low plant fibre intake (9) or too few test subjects (11) and the method used for the

estimation of transit time with radioopaque pellets (16) These pellets may perhaps stimulate intestinal peristalsis and thus blur the difference if any in the transit times between the control group and a group given a diet rich in fibre (17) Unlike some previous authors (26) we found good correlation between a shorter transit time and a higher frequency of bowel movements

The weight loss observed (Fig 3) may perhaps be explained by an increased caloric loss in the form of faecal fat (30) as the daily caloric intake remained unchanged throughout the experiment (Table II)

It has not been possible to demonstrate any hypocholesterolaemic effect of wheat bran (1 6 14) although this has been done for some of its biochemical components such as pectin (18 21) and lignin (25 31) The absence of such an effect of wheat bran accords with our results (Table I) On the other hand we were unable to confirm the previously described fall in serum triglyceride and serum calcium (14)

The method used in this study for measuring total 3 hydroxycholanic acid has been shown to reveal a postprandial increase in serum bile acid (29) This postprandial change was confirmed by LaRosso et al (24) using a sensitive radioimmunoassay technique and stated that conjugates of cholic acids (CCA) in serum are determined by the intestinal absorption of bile acids Therefore it should be possible to detect a change in bile acid absorption by measuring bile acids in serum

In an attempt to explain the unchanged serum level of total 3α hydroxycholanic acid three possibilities may be considered 1) that wheat bran does not bind bile acid in the intestinal lumen and consequently does not increase the excretion 2) that the binding if any and excretion of bile acids are so small that they do not change the amount of total 3α hydroxycholanic acid (the serum pool is only a small fraction of the total bile acid pool 8–10 mg in serum against 2–4 g in the total pool (28) 3) that two mutually independent bile acid circulations exist viz (*a*) an enterohepatic and (*b*) a serohepatic circulation that is to say that a change in one does not result in a change in the other

Patients with type IIa hyperlipoproteinemia a condition known to have a small pool size of cholic acid (10) have been found to have significantly lower serum CCA values than normal controls (23) presuming an inverse relationship to serum cholesterol However the relations betwen serum cholesterol and serum CCA were not further evaluated

The significance of the correlation observed between serum cholesterol and serum total 3α hydroxycholanic acid is as yet unknown (Fig 4)

The greater intake of calcium (Table II) was unexpected and reflected a greater intake of dairy products like milk yoghurt junket and cheese ($0\ 1 \geq p \geq 0\ 05$) The intake of vegetables was unaltered If this increase is associated regularly with bran intake there would be no reason to fear a diminished calcium absorption because of binding of calcium to phytine The fact that phytase in wheat bran is activated by such dairy sour products supports this point of view In a study of patients with diverticular disease (1) a small but significant reduction was found in the urinary excretion of calcium after bran However it could not be determined whether this phenomenon was due to a reduced intestinal absorption or to a reduced intake of calcium as no dietary analyses were performed

The correlation observed between the dietary cholesterol content and serum cholesterol lends support to theories advanced previously whereas the absence of a correlation between the other dietary components and serum lipids militates against them (20) The slight fall in sucrose intake thus does not result in any change in serum triglyceride

CONCLUSION

Our results show that it is possible to change the faecal flow pattern and intestinal transit time by an individual dosage of wheat bran even in subjects without major gastrointestinal symptoms At present we have no conclusive evidence to show that a reduction in transit time is desirable However the subjective improvement and weight loss obtained are in favour of an increase in the dietary plant fibre content in a population otherwise harassed by constipation and overweight

In the doses used in this study wheat bran did not affect the serum levels of cholesterol triglyceride calcium and 3α hydroxycholanic acid in young subjects

REFERENCES

1 Brodribb A J M & Humphreys D M Diverticular disease three studies Part III Metabolic effect of bran in patients with diverticular disease Br Med J 1 428 1976

2 Burk tt G P Walker A R P & Pa nter N S Effect of d etary f bre on stools and transit t mes and its role in the causat on of disease Lancet 2 1408 1972

3 Connell A M Sm th C L & Somsel M Absence of effect of bran on blood l pids Lancet 1 496 1975

4 Cowgill G R & Anderson W E Laxative effects of wheat bran and washed bran in healthy men JAMA 98 1866 1932

5 Cumm ngs J H D etary f bre Gut 14 69 1973

6 Durrington P W cks A C B & Heaton K W Effect of bran on blood l p ds Lancet 2 133 1975

7 Dyerberg J Lipoprote ner i plasma Thes s F a d l s Forlag Copenhagen 1973

8 Eastwood M A & Hamilton D Stud es on the adsorpt on of b le salts to non adsorped components of d et Bioch m B ophys Acta 152 165 1968

9 Eastwood M A Kirkpatrick J R M tchell W E Bone A & Hamilton T The effect of d etary sup plements of wheat bran and cellulose on faeces and colon c funct on Br Med J 4 39? 1973

10 Einarson K & Hellstrom K The formation of b le acids in patients w th three types of hyperl poproteinem a Eur J Cl n Invest 2 225 1972

11 Findlay I M Sm th A N M tchell W D Anderson A J B & Eastwood M A Effects of unprocessed bran on colon funct on in normal sub jects and in d vert cular d sease Lancet 1 146 1974

12 Hard nge M G & Stare F J Nutritional stud es of vegetarians 2 D etary and serum levels of choles terol J Clin Nutr 2 83 1954

13 Harvey R F Pomare E W & Heaton K W Effects of ncreased d etary f bre on ntest nal trans t Lancet 1 1278 1973

Heaton K W & Pomare E W Effect of bran on blood l pids and calc um Lancet 1 49 1974

Helms P The d et in Denmark 19 2 Ugeskr Laeger 137 2145 1975

16 H nton J M Lennard Jones J F & Young A C A new method for studying gut trans t t mes us ng rad opaque markers Gut 10 842 1969

17 Holm C N & Hansen L P Plantf bre and bowel transit t me Ugeskr Laeger 137 561 1975

18 Jenk ns D J A Leeds A R Newton C & Cumm ngs J H Effect of pectin guar gum and wheat fibre on serum cholesterol Lancet 1 1116 1975

19 Kessler G & Wolfman M An automated procedu e for the s multaneous determ nat on of calc um and phosphorus Clin Chem 10 686 1964

20 Keys A Anderson J T & Grande F Pred ction of serum cholesterol responses of man to changes n fats in the d et Lancet 2 959 1957

21 — F ber and pect n in the d et and serum choles ero concentration in man Proc Soc Exp B ol Med 106 555 1961

?2 K rwan V O Smith A N McConnell A A Mitchell W D & Eastwood M A Act on of d ffer ent bran preparat ons on colon c funct on Br Med J 4 187 1974

23 Korman M G Ellefson R D & Hofmann A F Fasting serum b le acid levels in the primary hyperl poproteinemias Mayo Cl n Proc 50 76 1975

?4 LaRosso N F Korman M G & Hoffmann A F Intestinal absorption The major determ nant of serum b le ac ds in pat ents with normal l ver function (abstract) Gastroenterology 67 806 1974

25 I indner P & Moller B Lign n A cholesterollower *ing agent Lancet 2 1259 1973*

26 Payler D K Pomare E W Heaton K W & Harvey R F The effect of wheat bran on ntest nal transit Gut 16 209 1975

27 Pearson S Stern S & McGavack T H A rap d accurate method for the determ nat on of total cholesterol in serum Anal Chem 25 813 1953

28 Peterslund N Personal commun cat on

29 Schwarz H P Bergmann K V & Paumgartner G A simple method for the est mat on of b le ac ds n serum Cl n Ch m Acta 50 197 1974

30 Southgate D A T & Durn n J V G A Calon conservat on factors An experimental re assessmen of the factors used in the calculat on of the energy value of human d ets Br J Nutr 24 517 1970

31 Th ffault C Belanger M & Poul ot M Tra tement de l hyperl poproté nem e essent elle de type ? par un nouvel agent thérapei tique la cellul ne Can Med Assoc J 103 165 1970

Acta Med Scand 202 131–133 1977

Proteinuria Following Renal Arteriography

Report of Two Cases

Lars Tejler Mats Ekberg Torsten Almen and Stig Holtås

From the Departments of Clinical Chemistry Medicine (Renal Unit) and Diagnostic Radiology University of Lund Malmo General Hospital Malmo Sweden

ABSTRACT Two patients reacting with transitory massive proteinuria after diagnostic renal arteriography with a commonly used non ionic contrast medium metrizoate are described One of them developed temporary renal failure the concentration of urinary albumin reached 330 g/g creatinine It is suggested that intratubular precipitation of proteins obstructing urinary flow might be one factor in the development of her renal failure

Since the introduction of the triiodinated ionic contrast media in general use today renal arteriography has been considered virtually without risk (1) Reports of serious renal complications are scarce and usually point to some predisposing factor such as dehydration myeloma renal artery stenosis diabetes mellitus liver disease or renal insufficiency (2 11 12 14 16 17) Yet within one month we observed two patients with no obvious predisposing factors who developed massive proteinuria following renal arteriography One of these patients displayed temporary renal failure

CASE REPORTS

Case I

A non parous 36-year-old woman with bleeding tendency (mainly melaena and haemoptysis) since the age of 19 had been subjected to repeated investigations including various roentgendiagnostic and endoscopic examinations but no bleeding sources were demonstrated and no defects were found in the coagulation mechanism Her menses were regular and there was no obvious menorrhagia Macroscopic haematuria and proteinuria were first noted at the age of 34 An i v pyelography (IVP) revealed nothing abnormal nor did a cystoscopy Recurrent episodes of haematuria were treated intermittently with ε amino-caproic acid because of suspected fibrinolysis During this therapy the haematuria diminished or disappeared but repeated investigations in drug free intervals revealed no fibrinolysis

During an episode of continuous macroscopic haematuria 2 years later IVP was repeated with normal findings The patient was referred to the Renal Unit at Malmo General Hospital for further evaluation On admission the haematuria had disappeared Physical examination disclosed nothing abnormal BP was 130/80 mmHg Hb 141 g/l ESR 18 mm/h leucocytes 6.7×10^9/l with a normal differential count platelets 266×10^9/l serum creatinine 97.2 μmoles/l (normal range 60–100) Plasma protein analysis was normal except for a moderate increase in polyclonal IgM (3.0 g/l) which had been detected four months earlier (3.0 g/l) Repeated urinalyses were normal Urinary albumin was 0.012–0.022 g/g creatinine (normal range <0.025) ^{51}Cr EDTA clearance was 101–95 ml/min IVP was again normal

Having been discharged from the hospital the patient was readmitted 4 weeks later because macroscopic haematuria had again developed A bilateral selective renal arteriography was performed Isopaque Coronar® (metrizoate) was used as contrast medium total volume 70 ml The angiogram was considered normal and the patient showed no adverse reactions at the Department of Radiology After her return to the Medical Ward nausea and vomiting developed and BP fell transiently On the following day oliguria and massive proteinuria (urinary albumin 330 g/g creatinine) were noted The proteinuria was non selective and plasma proteins of high molecular weight including β-lipoprotein (mol weight $2\text{–}3\times10^6$) were demonstrated in the urine

On the second day after the arteriography the patient became anuric and complained of lumbar pains Plasma protein analysis disclosed changes compatible with nephrosis i e hypoalbuminaemia hyperfibrinogenaemia and reduction of Ig concentrations (IgM was now 2.2 g/l) After mannitol loads (2×100 ml mannitol 25% w/v) urine production returned to normal Urine osmolality was low (125–205 mosmol/kg) and serum creatinine rose to a maximum of 698 μmoles/l on the seventh day with a corresponding fall in endogenous creatinine clearance to 21 ml/min Urinalysis disclosed microscopic haematuria and the presence of granular and waxy

A series of ^{131}I hippuran renograms was obtained on the second postarteriographic day there was normal parenchymal function bilaterally but a markedly prolonged passage time in the right kidney and a total outflow obstruction in the left These changes remained for two weeks Then partial normalization of the passage time was observed After another two weeks the renogram was completely normal

Nephrotomography ten days after the arteriography showed that both kidneys had increased in size compared with the IVP performed one month earlier Analysis of the serum complement system (C3 C4 Clq C5 and CH_{50}) gave normal results Repeated urine cultures were negative Fibrin/fibrinogen degradation products (FDP) in urine (6) were detected during the first postarteriographic week Thereafter repeated tests were negative There was a rapid fall in urinary albumin concentration and normalization was complete within 3 weeks Serum creatinine fell successively and was normal within 2 months when creatinine clearance values also had returned to normal (119 ml/min)

The patient has now been followed up for two years Her renal function has remained normal and no proteinuria has been observed There have however been some episodes of macroscopic haematuria and haemoptysis both of which can probably be explained by the recent discovery of a platelet defect i e the platelets react like those in hereditary thrombasthenia Intermittent antifibrinolytic treatment has been given without inadvertent effects but also without effect on the haematuria

Case 2

A 44-year old woman was admitted to the Renal Unit ter a small cyst had been seen at the inferior pole of one during an operation for uterine myoma Polycystic ase of the kidney was suspected as three of her had died at an early age in renal disease the ature of which could not be ascertained from the very scanty data available In her childhood the patient had experienced an episode of haematuria proteinuria and granular casts in the urine During her two pregnancies there was some temporary proteinuria Otherwise she had been well with no signs of renal disease

Physical examination on admission revealed normal findings BP was 140/80 mmHg Hb 132 g/l ESR 8 mm/h platelet and leucocyte counts normal serum creatinine 79 6 *μmoles/l Urinalysis revealed slight microscopic* haematuria with 10–15 erythrocytes per high power field An Addis count confirmed haematuria and in addition revealed a slight decrease in concentration ability Urinary albumin was 0 013–0 055 g/g creatinine ^{51}Cr EDTA clearance was 108–102 ml/min ^{131}I hippuran renography was normal No defect was found in the coagulation mechanism

Two IVPs were performed without any evidence of polycystic disease but since there were some difficulties in delineating the lower pole of the left kidney an aortography and a bilateral selective renal arteriography were performed and disclosed normal findings Isopaque Coronar® (metrizoate) was employed as contrast medium (total volume 180 ml) There were no immediate subjective adverse reactions Massive albuminuria (9 g/g creatinine) was observed on the following day Serum creatinine and endogenous creatinine clearance 2 days later were normal Unfortunately urinary albumin was not monitored

The patient was followed up for one year Serum creatinine remained normal and neither proteinuria nor haematuria were observed

DISCUSSION

The mechanism(s) behind the development of renal failure in some patients after renal arteriography is not known Some nephrotoxic effect of the contrast media seems likely (15) In dogs, acute tubular necrosis has been induced by aortic injection of various contrast media (10) In rabbits necrosis of tubular cells has been demonstrated following selective renal arteriography with metrizoate as contrast medium (5)

Opposing views are held on the role of renal ischaemia in the development of postarteriographic renal failure (7 19) Dean et al (4) attributed renal injury to increased blood viscosity secondary to effects on the erythrocytes caused by the contrast media

Contrast agents sometimes induce precipitation of Tamm Horsfall mucoprotein within the renal tubules (3) Acute renal failure following IVP in patients with myelomatosis is probably caused by intratubular protein precipitation (8 9 13) Transitory postarteriographic proteinuria was recently found in 25 of 28 urological patients referred for diagnostic renal arteriography concentrations of urinary albumin exceeding 10 g/g creatinine were found in 9 of them (18) Although no cases of renal failure were noted in that series we suggested that massive proteinuria may play some role in the development of postarteriographic renal failure

Our two patients showed no signs of diabetes myelomatosis liver disease or vascular disease Glomerular function as judged from ^{51}Cr EDTA clearance was normal in both patients before arteriography Clinically they were not dehydrated The arteriographic procedure was uneventful in both patients They received a commonly used contrast medium in doses generally employed in renal arteriography today

Both patients developed massive proteinuria after the arteriography In patient I the effect of the arteriographic procedure on the glomerular sieve system was profound as evidenced by the occurrence of very high molecular weight plasma pro-

teins in urine In view of the extreme proteinuria en countered the abundance of urinary casts and the findings in the renographies it seems reasonable to ascribe the ensuing renal failure at least in part to tubular blockage caused by protein precipitates This would thus be analogous to the renal failures in cases of myelomatosis referred to above

The presence of FDP in the urine of patient 1 could be explained in various ways It might initially merely reflect glomerular leakage of intact fibrinogen but the possibility also exists that the urinary FDP at least in part originate from fibrin deposits in the injured glomeruli In patient 1 the deranged glomerular function dominated but tubular function was probably affected too judging from the decreased urine osmolality In both patients the glomerular injury caused by the arteriographic procedure was reversible and no permanent renal dysfunction has been detected during the follow up periods

In view of the high incidence of postarteriographic proteinuria referred to above (18) and the risk of formation of intratubular protein precipitates obstructing urinary flow we believe that all patients referred for renal arteriography should be well hydrated and adequate diuresis established prior to renal arteriography to reduce intratubular protein concentrations We are aware that the contrast media alone cause an osmotic diuresis but this is of very short duration We also recommend repeated analysis of urinary albumin in the postarteriographic period In cases with pronounced albuminuria the patient should be closely supervised the glomerular function evaluated and urine volumes monitored Mannitol administration might be of value as it probably was in our patient 1

REFERENCES

1 Abrams H Angiography 2nd ed p 22 Little Brown and Company Boston 1971
2 Ansari Z & Baldwin D S Acute renal failure due to radio-contrast agents Nephron 17 28 1976
3 Berdon W E Schwartz R H Becker J & Baker D H Tamm Horsfall proteinuria Radiology 92 714 1969
4 Dean R E Andrew J H & Read R C The red cell factor in renal damage from angiographic media JAMA 187 27 1964
5 Evensen A & Skalpe I Cell injury and cell regeneration in selective renal arteriography in rabbits Invest Radiol 6 299 1971
6 Hedner U Ekberg M & Nilsson I M Urinary fibrin/fibrinogen degradation products (FDP) and glomerulonephritis Acta Med Scand 195 81 1974
7 Killen D & Owens G Changes of renal oxygen availability during Urokon and Hypaque aortography Ann Surg 152 957 1960
8 Lasser E C Dynamic factors in roentgen diagnosis 1st ed pp 49 110 Williams & Wilkins Baltimore 1967
9 Lasser E C Lang J H & Zawadski Z A Contrast media Myeloma precipitates in urography JAMA 198 945 1966
10 Lasser E C Lee S H Fisher E & Fisher B Some further pertinent considerations regarding the comparative toxicity of contrast materials for the dog kidney Radiology 78 240 1962
11 McEvoy J McGeown M G & Kumar R Renal failure after radiological contrast media Br Med J 4 717 1970
12 Older R Miller J Jackson D Johnsrude I & Thompson W Angiographically induced renal failure and its radiographic detection Am J Roentgenol 126 1039 1976
13 Perillie P E & Conn H O Acute renal failure after intravenous pyelography in plasma cell myeloma JAMA 167 2186 1958
14 Port F K Wagoner R D & Fulton R E Acute renal failure after angiography Am J Roentgenol 121 544 1974
15 Sacton H M Review article urography Br J Radiol 42 321 1969
16 Sidd J & Decter A Unilateral renal damage due to massive contrast dye injection with recovery J Urol 97 30 1967
17 Stark F & Coburn J Renal failure following methylglucaminediatrizoate (Renografin) aortography Report of a case with unilateral renal artery stenosis J Urol 96 321 1966
18 Tejler L Almen T & Holtås S Proteinuria following nephroangiography Acta Radiol In press 1977
19 Thomson H S Margolis G Grimson K S & Taylor H M Effects of intraarterial injection of iodine contrast media on the kidney of the dog Arch Surg 74 39 1957

Acta Med Scand 202 135–138 1977

Tachycardia, Hypertension and Syncope

A Case Report

Ingolf Nielsen Ole Pedersen Bjergaard Anders Tybjærg Hansen and Joseph Welner

From Medical Department B Rigshospitalet and the Department of Psychiatry Kommunehospitalet Copenhagen Denmark

ABSTRACT A case of orthostatic syncope with tachycardia and hypertension is described Initially the condition was interpreted as a dysfunction in the neurovascular orthostatic regulation, but extensive physiologic examinations failed to give a comprehensive explanation A psychiatric examination demonstrated the condition eventually to be hysteriform and the patient was completely cured by psychotherapy

Orthostatic hypotension is a well known cause of syncope In the following we will describe a case of orthostatic fainting associated with increased blood pressure Immediately after attaining standing position the patient felt prodromes to syncope and after standing for 1–4 min she actually fainted Concomitantly with these symptoms the heart rate increased from 55 to 170 and the BP from 110/70 to 170/120

CASE REPORT

The patient a 26-year-old woman was admitted to our department in March 1970 one year after the start of symptoms An aunt had cryptogenetic epilepsia otherwise there was no significant family history of disease The patient s childhood and adolescence had been normal and she had been in hospital only once for cerebral concussion at the age of 14 Since then she had suffered from episodes of headache 1–2 times a month She gave birth to a normal child in 1965

In 1969 she was admitted to a gynecological department for abdominal pains After conservative treatment for four weeks without improvement an explorative laparotomy was performed The only abnormal finding was a benign cyst the size of a walnut in the right ovary

One week after discharge the patient was readmitted with abdominal pains now associated with dizziness and syncope No obvious explanation of the symptoms was found and the patient was discharged without treatment Her condition was tolerable for two months but the symptoms did not subside completely eventually they increased and she was admitted to a medical department During this admission and subsequent admissions to a neurological department the patient was investigated thoroughly The only abnormal findings were syncopes associated with tachycardia (heart rate approximately 170) always provoked by upright posture BP was normal in the supine position but increased to clearly hypertensive values (170/120) at 60° tilting Treatment with etilefrin chloride (NFN) (Effortil®) fluorhydrocortisone acetate (NFN) (Florinef®) and phenytoin was tried successively and in vain

In the following months the condition deteriorated and at the time of admission to our department the patient had been bedridden for nine months She had been examined by a psychiatrist twice and found to be thoroughly normal without signs of hysteric constitution

RESULTS

On admission to our department the general condition of the patient was good Her nutritional state was normal and the musculature was well developed Generally speaking only the equinovarus position of the feet indicated that the patient had been restricted to bedrest for a long period She was a pleasant looking buxom young woman She seemed to be adequately concerned about her invalidity and her somewhat appealing attitude was likewise found to be within a normal reaction pattern The findings at routine clinical examination including neurological examination were normal It was impossible for the patient to raise the head from horizontal position without feeling dizzy with slightly blurred vision Attempts to assume the sitting or standing position led within seconds to syncope When again in level position the patient quickly regained consciousness

Routine laboratory tests did not disclose any

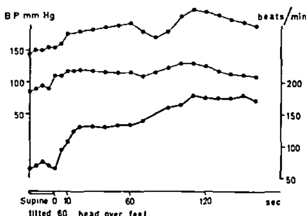


Fig 1 Systolic and diastolic BP (the two upper curves) and heart rate (the lower curve) during a representative tilt experiment

abnormality X ray of the chest and ECG were normal The creatinine clearance serum electro lytes thyroxin in plasma excretion of vanillyl mandelic acid and hydroxyindolyl acetic acid were normal The glucagon test was normal BP in the supine position was 110/70 and the pulse rate was 55–65 EEG and PEG gave rise to a certain suspi cion of cerebral disease Therefore a thoracic aortography of the internal carotid artery was erformed The aim was to visualize the vessels of neck and the greater cerebral arteries These stigations did not reveal anything abnormal e repeated EEG recordings performed during hospitalization did not sustain the suspicion of epilepsia or focal cerebral pathology

Tilting experiments

The patient was placed on a tilting table and tilted 60° head over feet BP was continually recorded by a needle in the brachial artery connected to an electronic transducer The pulse rate was recorded by ECG Fig 1 depicts a representative experi ment The main part of the increase in BP and pulse rate took place during the first 10 sec in the tilted position After 1 min the values had attained a plateau—BP 160–180/110–120 pulse rate 160–190 —where they remained even if the experiment was extended to 30 min in the tilted position

Cardiac output was normal in the supine position (5 3 l/min) and during 60° tilting it showed the nor mal decrease (4 3 l/min) On returning to the hori zontal position BP and pulse rate reached the initial values in 2–3 min The main part of the decrease took place in the first 20 sec in the supine position

Concomitant with the increase in pulse rate and BP the level of consciousness was lowered A coarse muscular tremor started The muscular tone was reduced The patient seemed frightened with a pale face dilated pupils and universal pronounced perspiration

In some of the tilting experiments the patient completely lost consciousness after a few minutes in the tilted position In other experiments the level of consciousness fluctuated but was always consid erably lowered The patient felt tilting to be ex tremely unpleasant when she was not completely unconscious In this state she understood simple questions but seemed too fatigued to respond The above mentioned symptoms were always en countered concomitant with increases in pulse rate and BP and were never seen when these values were normal The elevations of pulse rate and BP could only be provoked by tilting During tilting the respiratory frequency rose from about 15 to about 35/min No change in arterial CO_2 tension was found

Tilting in a g suit or submersion to the clavicle in a bath tub did not reduce the increases in pulse rate and BP Consequently it was concluded that the reaction was triggered by receptors in the arteries rather than in the low pressure part of the circula tion EEGs recorded during 60° tilt were completely normal and unchanged by tilting This result was difficult to explain considering the loss of con sciousness during tilting

Once more the possibility of a hysteric genesis of the condition was discussed but the efficacy of an i v injection of 5 mg propranolol during 60° tilting quenched the debate (Fig 2) In the course of 1 min a considerable decrease in pulse rate and BP took place The values remained at the lower level dur ing protracted tilting Cardiac output decreased from 4 3 l/min to 2 1 l/min Concomitant with the changes in pulse rate and BP the usual discomfort experienced by the patient during tilting completely vanished When propranolol 5 mg i v was ad ministered before tilting the increase in pulse rate and BP in the erect position could be almost completely inhibited In another 60° tilting experi ment when 5 mg phentolamine was administered i v the BP decreased to supine values in the course of 5 min while the pulse rate remained high The cerebral condition of the patient was considerably ameliorated after injection of phentolamine but palpitational discomfort persisted

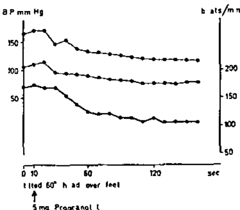


Fig 2 Systolic and diastolic BP (the two upper curves) and heart rate (the lower curve) after i v injection of 5 mg propranolol in the tilted position

Isoprenaline was infused i v (at steady state 3–4 μg/min) with the patient in the supine position She developed a pronounced tachycardia Cardiac output increased considerably The patient experienced a similar pronounced discomfort as during tilting

Based on the clearcut effect on the symptoms of i v propranolol in the tests oral propranolol treatment was initiated in a dosage of 10 mg q i d increasing to 40 mg q i d In the course of weeks the patient was fully mobilized She was then given placebo and after three days the symptoms recurred completely The propranolol treatment was started again and the symptoms disappeared once more in a few days At discharge the orthostatic reaction of heart rate and BP was completely normal and no discomfort persisted in standing position At this point we found that the condition had proved to be a somatic dysfunction although not at all well understood The working hypothesis was abnormal orthostatic cardiovascular reflexes which in their turn did not manage by negative feed back to inhibit the excessive reaction

Two months later the symptoms reappeared and the patient was admitted once more Tilting experiments revealed only slight increases in pulse rate and BP but the patient lost consciousness as before propranolol treatment An estimation of total cerebral blood flow by the xenon wash out technique was performed in supine and tilted position A normal blood flow was found in both conditions After these findings a hysteric genesis was found to be the most likely explanation of the condition and propranolol treatment was discontinued A tilting experiment in somnolent state induced by pentymal injection showed no abnormal pulse and BP reaction This was considered to be final proof of the mental origin of the condition and suggestive therapy was initiated by means of daily sessions with diathermy and a gradual increase in the tilt of the bed Concomitant physical treatment was started and the patient was told over and over again that this combined effort would cure her In the course of one month she was fully mobilized and discharged

When the patient s husband learned that the condition was hysterical he immediately filed a divorce The patient eventually remarried and now five years later is evidently leading a normal life

DISCUSSION

The clinical course has demonstrated that the condition was of hysterical origin The question arises could the diagnosis have been made earlier—before all the elaborate and expensive physiological measurements were made? In hindsight the answer of course is yes However the lesson to be learned from this case is we think that the modern medical establishment with its physiological approach is inclined to proceed as we did when faced with a problem patient with impressive physiological attributes and no obvious neurotical habitus While we consider that the overall procedure was reasonable given the modern medical institutions the correct diagnosis should have been reached earlier

The outcome of the propranolol injection test the subsequent result of propranolol treatment and the effect of the single blind placebo period were instrumental in our first conclusion that the condition was somatic This conclusion is unwarranted The β blocker is certainly able to inhibit an increase in heart rate irrespective of mechanism It must be assumed that this patient had created her hysterical manifestations around the normal orthostatic pulse rate reaction gradually increasing it Beta blockade removed an essential part of the hysterical reaction pattern and thereby—for a time—impeded the whole reaction In due time the patient learned to circumvent the block and restore her performance

The hysterical neurotic expresses her conflict through somatic channels more or less subtle Interference with these channels can change the clinical picture for a time but will not cure the patient

Accordingly the outcome of the propranolol medication should not have brought the investigation to a temporary halt The intrigueing normality of the EEG during tilting should have led us to measure cerebral blood flow at this time It was measured later and revealed the true nature of the condition loss of consciousness in absence of reduced cerebral blood flow cannot be a true condition when epilepsia has been ruled out

Acta Med Scand 202 139–144 1977

Combination Chemotherapy of an APUDoma

With Special Reference to the Therapeutic Value of Monitoring Hormonal Substances

Mogens Hansen Heine H Hansen O Paaske Hansen and Bo Hainau

From the Department of Internal Medicine C Bispebjerg Hospital and the Department of Pathology Finsen Institute Copenhagen Denmark

ABSTRACT Investigations are presented on the occurrence of tumour products during combination chemotherapy of a 49 year-old female with an APUDoma metastatic to the liver Calcitonin was demonstrated in high concentration in the tumour tissue Serum calcitonin, serum histaminase and 5 HIAA in a 24 hour urine sample increased immediately after the administration of cytotoxic agents falling subsequently below the pretreatment level These findings indicate a therapeutic effect with lysis of tumour cells Continuous determination of the three tumour substances showed an increase in these products before clinical suspicion of progression Electron microscopic examination during the initial course disclosed the tumour to be an APUDoma Autopsy failed to disclose a primary site outside the liver Further autopsy findings were an adenoma of the thyroid and a chromofobe adenoma of the pituitary thus assigning the patient to type I multiple endocrine neoplasia

Basically through the studies of Pearse (23) a number of apparently unrelated cells situated in endocrine as well as non-endocrine tissues have been found to share common cytochemical and ultrastructural features This cell system is usually designated APUD according to its basic characteristics (amine content and/or amine precursor uptake and amino acid decarboxylation) and it is possibly derived from the neural crest (25) A wide range of endocrine tumours may develop corresponding to these widely scattered neuroendocrine cells partly as multiple endocrine neoplasia partly as solitary tumours—APUDomas (26)

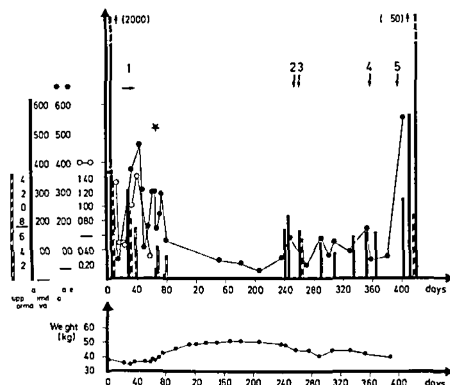
Apart from amine turnover a characteristic feature of APUDomas is polypeptide production The polypeptide calcitonin is found elevated in serum and/or has been demonstrated in tumour tissue in several cases of APUDomas (18)

In medullary carcinoma of the thyroid calcitonin is a useful marker substance in the diagnosis of familial cases (1 29 30) and in the detection of occult tumour tissue following surgery (13) In a similar fashion determination of amines in serum and urine is important in the diagnosis of other APUDomas such as carcinoids and pheochromocytomas So far these hormonal markers have been used mainly in connection with surgical management while there has been little information on decreased hormonal levels following medical therapy and exclusively in pancreatic islet cell carcinoma producing pancreatic polypeptide hormones (9 12 20 28)

The subject of this report is the value of determining tumour substances during combination chemotherapy of a patient with an APUDoma

CASE REPORT

The patient a 49 year-old woman previously in good health was admitted in Nov 1973 because of sustained dyspepsia with concomitant anorexia and a weight loss of 10 kg Gastrointestinal X rays gastroscopy and a percutaneous liver biopsy revealed no evidence of neoplasia However because of an additional weight loss of 10 kg an exploratory laparotomy was performed in Feb 1974 The liver was enlarged studded with several small tumours Careful investigations of the gallbladder stomach intestines pancreas and internal genital organs showed no abnormalities



F g 1 We ght and course c hormonal substances
1 cl nical mprovement
2=f rst cl n cal progress on
3=second chemo herapy programme n t a ed
4=second cl n cal progress
*at day 66 corresponds to day 1 in F g 2

croscop cal exam nat on of a b opsy from the l ver les gave rise to a susp cion of APUDoma later con ed by electron m croscopy

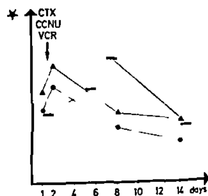
Subsequently comb nat on chemotherapy was init ated includ ng cyclophospham de (CTX) 1 (2-chloroethyl) 3-cyclohexyl 1 n trosourea (CCNU) v ncrist ne (VCR) and methotrexate (MTX) in four week cycles The schedule cons sted of CTX 700 mg/m² i v VCR 1 3 mg/m² i v and CCNU 70 mg/m² by mouth all on day 1 plus MTX 20 mg/m² by mouth on days 18 and 21 In the f rst cycle VCR was given on days 10 and 24 too In the th rd and fourth cycles the doses of CTX CCNU and MTX were reduced because of leukopen a and stomat t s VCR was w thheld n cycles four to s x because of paraesthes ae

During th s modal ty of therapy anorexia d sappeared the pat ent ga ned in we ght and the l ver d m n shed n size (Fig 1) On the 80th day of treatment laparoscopy was performed The l ver appeared sl ghtly enlarged w th small wh tish tumour nodules B ops es from the nodules as well as from normal look ng l ver t ssue were taken for *determinat on of calc ton n On the 86th day of treatment* the patient was d scharged for cont nued treatment on out pat ent bas s

E ght months after inst tut on of treatment we ght loss was recorded aga n Hormonal substances were in creased suggest ng progress on of the d sease Conse quently the comb nat on chemotherapy programme was changed to adriamyc n (ADM) and procarbaz ne (PCB) n three week cycles The dose of ADM was 35 mg/m² v on day 1 and of PCB 35 mg/m² by mouth da ly on d y 1 7 On th s med cat on the pat ent mpro ed and he we ght was almost stable for three months Further pro gress on was then obv ous w th we ght loss and enlarge ment of the l ver The cond t on deteriorated she wa adm tted 405 days after commencement of herapy an d ed ? weeks later

B ochem cal stud es

In t ally serum calc ton n (I) serum h stam nase (diam n ox dase) (31) 5 hydroxy ndoleacet c acid (5 HIAA) an



F g 2 Course of hormonal substances at adm n ra t on of cytotox c agents on day 1 ●=cal ton n ▲=h tam nase ▬=5 hydroxy ndoleacet c ac d

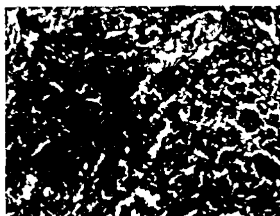


Fig 3 Light micrograph of liver tumour The small elongated tumour cells are arranged in solid strands separated by collagen Part of the tumour is necrotic Magnification ×51

catecholamines in the 24 hour urine were elevated (Fig 1)

During treatment calcitonin in serum reached its lowest value on day 79 At this time biopsies from the liver nodules showed a calcitonin value of 5 900 ng/g wet wt while no detectable calcitonin was present in normal liver tissue (analysis performed by the Stockholm Immune Laboratory)

Initially 5 HIAA was markedly increased catecholamines only slightly These substances reached normal levels during the initial treatment (Fig 1) It is however noteworthy that the substances changed during treatment synchronously with changes correlated to the administration of CTX CCNU and VCR

Following the third course of CTX CCNU and VCR (* in Fig 1) calcitonin histaminase and 5 HIAA were monitored more closely (Fig 2) All three substances increased immediately and subsequently fell below pretreatment levels The histaminase analysis was performed by N Tryding Kristianstad Sweden At the time of the first progression pentagastrin stimulation was performed (14) without any rise in calcitonin levels

Plasma glucagon serum insulin serum gastrin serum PTH VMA homovanillic acid and ketogenic steroids in the 24 hour urine sample were normal Growth hormone was decreased but showed a paradoxical rise after i v administration of glucose Serum calcium and phosphate were normal at several determinations

Pathological findings

Light microscopy of the liver biopsy demonstrated an invasive tumour composed of elongated or spindle shaped cells arranged in solid strands separated by collagen tissue (Fig 3) Congo red reaction for amyloid was negative Part of the biopsy was fixed in aldehyde-dichromate-OsO_4 according to Pearse (24) embedded in Epon sectioned and stained for electron microscopy using standard techniques and examined in a Philips EM 301 instrument All tumour cells contained cytoplasmic granules 90-160 nm in diameter The granules had an electron-dense core surrounded by a translucent zone and were limited peripherally by a membrane (Fig 4) Amyloid was not found These findings were interpreted as consistent with the presence of a malignant tumour originating from the APUD system At the post mortem examination the liver was extensively infiltrated with tumour tissue Small metastases microscopically identical to the liver tumour were present in the mesencephalon and hypothalamus The thyroid gland contained a small foetal adenoma and the pituitary gland a chromophobe adenoma No tumour tissue was found elsewhere

DISCUSSION

This patient had a malignant tumour of the liver with metastasis to the brain The presence of cytoplasmic granules in all tumour cells exhibiting the same ultrastructural features as the neurosecretory granules in APUD cells strongly suggested that the tumour originated from cells belonging to this system (8)

Apart from brain metastases no malignant tumour tissue was found outside the liver The question remains open whether the liver tumour was primary or metastatic from an unknown primary site the autopsy findings favouring the first possibility The concomitant presence of adenomas in the thyroid and the pituitary indicates a case of multiple endocrine neoplasia of type I (5 10)

Under normal circumstances many APUD cells are engaged in the production of polypeptides and amines while APUDomas may have the capacity of producing both physiological and other phylogenically related substances of the APUD system (26) In this case the tumour seems to have produced calcitonin serotonin and histaminase (and maybe catecholamines) The presence of calcitonin in the tumour tissue was substantiated while assays for determination of histaminase and serotonin in the tumour tissue were not available However the almost parallel course of calcitonin histaminase and the metabolic product of serotonin 5 HIAA following lysis of tumour cells supports a tumour production of these substances (Fig 2) Similarly catecholamines may have been produced in some degree in the tumour judging from the concomitant course of catecholamines and 5 HIAA during treatment (Fig 1) However the excretion of catecholamines was not elevated more than might be explained by stress due to advanced disease On the other hand the phenomenon might be explained by a limited capacity of APUD tumour tissue to produce catecholamines (3 23 32)



Fig 4 Electron micrograph of liver tumour The tumour cells contain a large number of granules with an electron-dense core surrounded by a translucent zone and limited peripherally by a membrane (insert) Magnification ×7475 insert ×23400

The different tumour substances by themselves do not allude to a primary site of the tumour since calcitonin (10 11 16 17 18 27 32) as well as histaminase (3 6 7) and serotonin (15 17 19 22) have been observed in APUDomas of different sites

In this case tumour products showed changes of values according to the clinical course (Fig 1) During the initial period the 5 HIAA values decreased after administration of CTX CCNU and VCR with subsequent normalization Determination of 5 HIAA requires 24 hour collection of urine which may account for some variation in the values particularly when the urinary output is small as in this case A rapid and much more pronounced fall of calcitonin was observed during the initial treatment When the first progression was clinically suggested all tumour products were increased During the second remission which was only partial calcitonin levels were again significantly decreased and subsequently increased for at least one month before clinical evidence of the second and last progression while 5 HIAA evaluation was not conclusive until a rather late stage of the progression Except in the initial period histaminase was determined only twice but it is remarkable that all

histaminase values were changed according to the changes in calcitonin although only the first and the last histaminase values were above normal

These findings suggest a therapeutic value of frequent determination of hormonal substances during chemotherapy of APUDomas However the applicability of peripheral blood findings during chemotherapy depends on several conditions *Firstly* the substance or substances must be produced and secreted by the tumour cells in the primary tumour or in the metastases Measurements of arteriovenous differences or production and secretion by tumour cells in vitro may establish whether or not this is so but usually—as in this case—demonstration of hormonal substances in tumour tissue together with an otherwise unexplained abnormal elevation of peripheral values is the only applicable method *Secondly* the production and particularly the secretion and elimination rate have to be almost constant i e the peripheral values must correspond to the quantity of tumour cells These conditions appear to occur in multiple myeloma (21) but are at the moment only hinted in APUDomas In our experiment (Fig 2) all the tumour substances reached a lower level a few days after administration of cytostatic agents indicating regression of tumour which was also evidenced by a decrease in liver size However this particular experiment demonstrates that the tumour substances increase immediately after administration of cytotoxic agents During effective chemotherapy of acute lymphocytic leukemia and Burkitt s lymphoma too an increase in phosphate and potassium may occur extracellularly within the first two days after administration of cytotoxic agents suggesting rapid lysis of the tumour cells (2 33) Furthermore changes in urinary HCG according to the therapeutic schedule are known to occur during chemotherapy of choriocarcinoma (4) Thus this phenomenon should be taken into account when assessing the effectiveness of a chemotherapeutic programme and may reveal sensitivity or resistance at a very early stage *Thirdly* tumour cells that develop resistance to a current chemotherapy programme must produce and secrete the same substances as the primarily sensitive cells This seemed to occur in the present case suggesting that the tumour was monoclonal *Fourthly* the chemotherapeutic agents themselves must be without influence on the production and secretion of hormonal substances in non affected tumour cells

All these items call for further elucidation At present however there is no evidence against the reliability of hormonal substances as therapeutic tumour markers if errors in analysis and the time of determination are taken into account

ACKNOWLEDGEMENT

The work was supported by Esper and Olga Boel s Foundation

REFERENCES

1. Almqvist S Telenius Berg M & Wasthed B Serum calcitonin in medullary thyroid carcinoma Radioimmunoassay technique and diagnostic value Acta Med Scand 196 177 1974
2. Arseneau J C Bagley C M Anderson T & Canellos G P Hyperkalcemia a sequel to chemotherapy of Burkitt s lymphoma Lancet I 10 1973
3. Atkins F L Beaven M A & Keiser H R Dopa decarboxylase in medullary carcinoma of the thyroid N Engl J Med 289 545 1973
4. Bagshawe K D Immunological methods in the diagnosis and monitoring of tumours In Medical oncology (ed K D Bagshawe) pp 245–267 Blackwell Scientific Publications Oxford London Edinburgh and Melbourne 1975
5. Ballard H S Frame B & Hartsock R J Familial multiple endocrine adenoma—peptic ulcer complex Medicine 43 481 1964
6. Baylin S B Abeloff M D Wieman K C Tomford J W & Ettinger D S Elevated histaminase (diamine oxidase) activity in small-cell carcinoma of the lung N Engl J Med 293 1286 1975
7. Baylin S B Beaven M A & Keiser H R Serum histaminase and calcitonin levels in medullary carcinoma of the thyroid Lancet I 455 1972
8. Bensch K G Corrin B Pariente R & Spencer H Oat-cell carcinoma of the lung Cancer 22 1163 1968
9. Broder L E & Carter S K Pancreatic islet cell carcinoma Results of therapy with streptozotocin in 52 patients Ann Intern Med 79 108 1973
10. Cattan D Pappo E Dervichian D Mellière D Chelloul Y Calmettes C & Milhaud G Carcinoid tumour of the jejunum with thyrocalcitonin containing liver metastases associated to a benign C cell adenoma of the thyroid Arch Fr Mal App Dig 62 141 1973
11. Coombes R C Hillyard C Greenberg P B & Mac Intyre I Plasma immunoreactive-calcitonin in patients with non thyroid tumours Lancet I 1080 1974
12. Danforth D N Triche T Doppman J L Beazley R M Perrino P V & Recant L Elevated plasma proglucagon like component with a glucagon secreting tumor Effect of streptozotocin N Engl J Med 295 242 1976
13. Goltzman D Potts J T Ridgway E C & Ma

loof F Calcitonin as a tumour marker N Engl J Med 290 1035 1974

14 Hennessy J F Wells S A Ontjes D A & Cooper C W A comparison of pentagastrin injection and calcium infusion as provocative agents for the detection of medullary carcinoma of the thyroid J Clin Endocrinol Metab 39 487 1974

15 Horai T Nishihara H Tateishi R Matsuda M & Hattori S Oat-cell carcinoma of the lung simultaneously producing ACTH and serotonin J Clin Endocrinol Metab 37 212 1973

16 Kaplan E L Arnaud C D Hill B J & Peskin G W Adrenal medullary calcitonin like factor A key to multiple endocrine neoplasia type 2 Surgery 68 146 1970

17 Kaplan E L Sizemore G W Peskin G W & Jaffe B M Humoral similarities of carcinoid tumours and medullary carcinomas of the thyroid Surgery 74 21 1973

18 Milhaud G Calmette C Taboulet J Julienne A & Moukhtar M S Hypersecretion of calcitonin in neoplastic conditions Lancet 1 462 1974

19 Moertel C G Beahrs O H Woolner L B & Tyce G M Malignant carcinoid syndrome associated with non-carcinoid tumours N Engl J Med 273 244 1965

20 Murray Lyon I M Eddleston A L W F Williams R Brown M Hogbin B M Bennett A Edwards J C & Taylor K W Treatment of multiple hormone producing malignant islet-cell tumour with streptozotocin Lancet 2 895 1968

21 Nathans D Fahey J & Potter M The formation of myeloma protein by a mouse plasma cell tumor J Exp Med 108 121 1958

O Neal L W Kipnis D M Luse S A Lacy P E & Jarrett L Secretion of various endocrine substances by ACTH secreting tumours—gastrin melanotropin norepinephrine serotonin parat hormone vasopressin glucagon Cancer 21 1219 1968

23 Pearse A G E Common cytochemical and ultrastructural characteristics of cells producing polypeptide hormones (the APUD series) and their relevance to thyroid and ultimobronchial C cells and calcitonin Proc R Soc B 170 71 1968

24 — Histochemistry Theoretical and applied p 1457 Churchill Livingstone Edinburgh and London 1972

25 Pearse A G E & Polak J M Neural crest origin of the endocrine polypeptide (APUD) cells of the gastrointestinal tract and pancreas Gut 12 783 1971

26 — Endocrine tumours of neural crest origin Neurolophomas apudomas and the APUD concept Med Biol 52 3 1974

27 Silva O L Becker K L Primack A Doppman J & Snider R H Ectopic production of calcitonin Lancet 2 317 1973

28 Stadil F Stage G Rehfeld J F Efsen F & Fischerman K Treatment of Zollinger Ellison syndrome with streptozotozin N Engl J Med 294 1440 1976

29 Tashjian A H Howland B G Melvin K E W & Hill C S Immunoassay of human calcitonin N Engl J Med 283 890 1970

30 Telenius Berg M Almqvist S Berg B Hedner P Ingemansson S Tibblin S & Wästhed B Screening for medullary carcinoma of the thyroid in families with Sipple s syndrome Evaluation of new stimulation tests Eur J Clin Invest In press 1976

31 Tufvesson G & Tryding N Determination of diamine oxidase activity in normal human blood serum Scand J Clin Lab Invest 24 163 1969

32 Voelkel E F Tashjian A H Davidoff F F Cohen R B Pertia C P & Wurtman R J Concentrations of calcitonin and catecholamines in pheochromocytomas a mucosal neuroma and medullary thyroid carcinoma J Clin Endocrinol Metab 37 297 1973

33 Zusman J Brown D M & Nesbit M E Hyperphosphatemia hyperphosphaturia and hypocalcemia in acute lymphoblastic leucaemia N Engl J Med 289 1335 1973

Acta Med Scand 202 145–147 1977

EDITORIAL

Radionuclides in Cardiology

More than 50 years ago Blumgart and Yens (2) first used radioactive tracers for studying the rate of blood flow in cardiac patients Their studies were performed almost 15 years before the first use of iodine isotopes in the study of the thyroid Nylin (5) in 1945 used blood corpuscles labeled with radioactive phosphorus in studies of the circulation Prinzmetal et al (7) in 1947 administered sodium 22 i v and with the aid of a Geiger counter placed over the precordium recorded a radiocardiogram from which evidence of shunting and cardiac output could be calculated Eleven years later radioiodinated serum albumin was used by Rejali et al (8) to image the cardiac blood pool for the detection of pericardial effusion

In the early 1960s Carr et al (3) developed myocardial imaging using cesium as the radio tracer They also used chlormerodrin whereas Malek et al (4) used iodinated tetracycline for detection of an infarcted area

Two methods are available for myocardial imaging Negative imaging of an infarcted area of the myocardium is produced by tracers which can also be used for the non invasive evaluation of regional perfusion These tracers follow the Sapirstein principle that is they are rapidly cleared from the blood and are concentrated in the organ under investigation Under these circumstances the regional distribution will be flow related To this category of tracers belong the monovalent cations of potassium rubidium cesium and thallium substrates of metabolism drugs and hormones offer other sources of compounds which might be utilized for myocardial imaging

Thallium 201 is the radionuclide most often used although it is expensive It accumulates in the normal myocardium Its distribution represents a function of the regional distribution of blood flow and of extraction over the cell membrane by the activity of the Na^+ K^+ ATPase system Thallium 201 is rapidly cleared from the blood it has a long physical half life of 73 5 h and a relatively low energy radiation and its maximal myocardial concentration is reached within a few minutes so that scintigraphy can be started 10 min after injection—all these advantages make thallium 201 the preferred radionuclide for negative myocardial imaging

A positive imaging of the myocardium is produced by tracers which accumulate in an infarcted area To this group belong the mercurials but their clinical usefulness is limited by the high radiation dose delivered when sufficient radionuclide is administered for satisfactory imaging results Tetracycline labeled with reduced technetium 99m could localize infarcts although a 24 hour delay following i v injection was usually required to allow for sufficient blood clearance In addition excessive hepatic uptake of tetracycline can make it difficult to localize the infarct The tracer most often used for positive myocardial imaging is technetium 99m labeled phosphate Both polyphosphate and pyrophosphate are in wide use in coronary care units

The major indications for radionuclides in cardiology is as an aid in the diagnosis of acute myocardial infarction and coronary insufficiency for determination of ventricular function and as a screening procedure for diagnosing ischemic and congenital heart disease

Acute myocardial infarction is still difficult to diagnose in several cases despite the valuable information yielded by the patient s history ECG and enzyme values In these doubtful cases a myocardial imaging is often of additional value Technetium 99m pyrophosphate is often used although results with glucoheptonate show promise results with technetium 99m EDTA have not proved satisfactory More frequent serial scintigraphy may be possible with indium 113m labeled ... nds

such as ^{113m}In EDTMP this compound seems to be sequestered by acutely infarcted myocardium as early as 4 hours after infarction

Most experience has been obtained with technetium 99m pyrophosphate which seems to produce a positive image in most cases of acute myocardial infarction Positive imaging requires some perfusion in the vicinity of the target site to transport the tracer from its injection Furthermore the tracer must be able to diffuse to the site of localization and the tracer should become fixed at the target site Absence of one or more of these prerequisites might explain some of the false negative findings as may technical difficulties in connection with imaging of the posterior wall

Two thirds of the patients with unstable angina show positive scintigrams These uptakes seemed to be spread more diffusely over the myocardial area compared with patients with transmural myocardial infarction and the intensity was lower It is not known whether these positive scintigrams reflect severe ischemia or small infarcts otherwise undetected Positive imaging in non ischemic heart patients may be produced by cytostatic agents such as doxorubicine and may be found in patients with cardiomyopathies Furthermore positive imaging has been reported in 14% of a consecutive series of bone scans performed on non cardiac patients (6) and in cardiac patients in a non acute stage of ischemic heart disease (1)

The interval from the onset of symptoms to a positive scintigram is usually said to be 12 hours The experience of the Malmo team indicates that in some patients a positive scintigram is obtained as soon as 4 hours after onset of symptoms If this short interval is confirmed in an extended material acute myocardial infarction can be diagnosed earlier than was possible previously and cardiac monitoring can be started early And if a negative scintigram excludes the diagnosis of infarction the patient can be discharged from the hospital early and expensive beds can be saved

Perfusion scintigraphy with thallium 201 has a high degree of accuracy in detecting an acute myocardial infarction This accuracy depends on the time of scintigraphy after onset of symptoms and on the size of the infarction According to some authors no false negative scintigrams are obtained if the patients are studied within 6 hours after onset of symptoms In the experience of the Malmo team some otherwise undetected perfusion defects were diagnosed when ^{99m}Tc pyrophosphate scintigraphy was combined with perfusion scintigraphy This number of patients was small and mainly restricted to those with involvement of the inferior myocardium an area which may be difficult to image by pyrophosphate

A perfusion defect obtained with thallium scintigraphy does not differ between an acute or old infarct On the other hand the positive imaging with ^{99m}Tc pyrophosphate usually disappears about 1? days after the onset of symptoms In this respect the two methods complement each other

Phantom studies with perfusion scintigraphs show that lesions are best defined when viewed either en face or in tangent and that a 3 5 cm transmural lesion could always be defined whereas a transmural lesion with a diameter of 2 5 cm was defined with greater difficulty and a 2 5 cm non transmural was not detected at all

Myocardial perfusion defects can be produced by diseases other than those involving the coronary arteries such as sarcoid and cardiomyopathies

The rest injected scan may be of diagnostic value with an equivocal history of angina pectoris and/or inconclusive ECGs due to bundle branch block or digitalis induced ST T changes

Patients with a clinically silent ischemic heart disease are usually diagnosed with the aid of a stress ECG This can however be normal in patients with coronary artery disease diagnosed with a coronary arteriography and a small but significant number of patients may have a false positive exercise test A stress injected scan may be of diagnostic value in these patients who at rest have a satisfactory flow in the coronary arteries resulting in a normal perfusion but when myocardial oxygen demands increase due to exercise pacing or drugs blood flow distal to a fixed stenosis may not increase to meet these demands while flow to the normal bed increases if tracer is administered at this time thus producing a difference in tracer concentration The scan will give information on the location and extent of the zone of abnormal perfusion Some studies indicate a greater sensitivity of the scan when compared with stress ECG in the detection of ischemic heart disease Even so rather pronounced coronary artery disease may exist with a normal stress scan

A further category of patients is those with a history and/or physical findings which could fit in with a congenital heart lesion with a shunt A radio-

cardiogram will make a more complicated and time consuming cardiac catheterization unnecessary Furthermore if a shunt exists it is possible by re peated measurements to follow the patient and find out whether an increase or decrease of the amount of shunted blood has occurred

Non invasive determination of ventricular func tion is a further indication for a blood pool tracer In the first pass study data can be recorded either with a scintillation camera and a computer or with a single probe placed over the left ventricle and an analogue recorder This examination takes a comparatively short time which is of value in criti cally ill patients and gives valuable hemodynamic information including the ejection fraction In the gated blood pool scan study a gate is used to trigger the scintillation camera in synchrony with the car diac cycle Usually but not necessarily the ECG is used The gate permits signals to pass only during selected portions of the cardiac cycle usually end systole and end diastole This technique also pro vides the ejection fraction but in addition it is pos sible to get information on the extent of regional wall motion of the left ventricle revealing dyski netic or akinetic areas Both the first pass and the gated scan technique correlate well with contrast angiography

The scintillation camera has admirably served the field of nuclear medicine and the recent mobile cameras make it possible to obtain detailed informa tion at the bedside from critically ill patients who cannot be moved from the ward However its use for myocardial imaging is encumbered by fun damental handicaps First the scintillation camera squeezes the image of a three dimensional object into a two-dimensional plane Second the field of view and resolution vary with depth which greatly complicates quantitative assessments New meth ods such as the emission computerized axial tomo graphy (CAT) have been developed to overcome these difficulties Emission CAT provides trans axial sectional images of structures that contain gamma ray emitting radionuclides Conceptually this method is identical to that used by the EMI scanner

Positron tomography is hampered by the neces sity of being close to a cyclotron and even the recent baby cyclotrons are expensive A new possibility has turned up with a germanium-67 generator from which gallium 68 is eluated and this is a positron emitter with a short physical half life Furthermore new pharmaceuticals such as myo cardial metabolic substrates labeled with nuclides akin to physiological processes may be of value for assessment of metabolism and ammonia for the as sessment of perfusion

Radionuclides have been used in cardiology for several years but it is not until the last few years that they have come into common practice Radio nuclides have already provided cardiology with non invasive detailed information also at the bed side in critically ill patients Their possible value in emergency wards might save expensive patient beds The latest improvements both in equipment and in pharmaceuticals indicate that radionuclides in cardiology will be a field in which much will hap pen in the near future

Bengt W Johansson
Department of Medicine Heart Section
Malmo General Hospital Malmo Sweden

REFERENCES

1 Ahmad M Dubiel J Logan K W Verdon T A & Martin R H Limited clinical diagnostic specificity of technetium 99m stannous pyrophosphate myocar dial imaging in acute myocardial infarction Am J Car diol 39/1 50 1977
2 Blumgart H L & Yens O C The rate of blood flow as determined by a new method Am J Physiol 72 216 1925
3 Carr E A Beierwaltes W H Patno M E et al The detection of experimental myocardial infarcts by photoscanning Am Heart J 64 650 1962
4 Malek P Vavrejn B Ratusky J & Kolc J Detec tion of myocardial infarction by in vivo scanning Cardiologia 51 22 1967
5 Nylin G Dilution curve of activity in arterial blood after intravenous injection of labeled corpuscles Am Heart J 30 1 1945
6 Prasquier R Taradash M R Botvinick E H Shames D M & Parmley W W The specificity of the diffuse pattern of cardiac uptake in myocardial in farction imaging with technetium 99m stannous pyro phosphate Circulation 55/1 61 1977
7 Prinzmetal M Corday E Bergman H C et al Radiocardiography a new method for studying the blood flow through the heart in human beings Science 108 340 1948
8 Rejali A M Macintyre W J & Friedell H L A radioisotope method of visualization of blood pools Am J Roentgenol 79 129 1958

BOOK REVIEW

Bronchial asthma Mechanisms and therapeutics Edited by E B Weiss and M S Segal 1168 pages illustrations and indexed US$ 50 00 Little Brown and Co Boston Mass 1976

The groups of diseases causing airflow obstruction has attracted a great deal of interest in recent years when discussion on tobacco smoking and other air pollution has been current Increased awareness of the importance of such diseases has stimulated clinical and experimental research New knowledge within immunology physiology pharmacology and other basic medical fields has rapidly been applied to diagnostics and therapeutics and even the specialist has had difficulty in following progress in these fields

This volume provides good guidance to anybody interested in following new trends in research on asthma and their clinical significance It is edited by E B Weiss Associate Professor of Medicine at the University of Massachusetts Medical School and M S Segal Emeritus Professor of Medicine at Tufts University School of Medicine They have attracted a group of highly qualified contributors many of them outstanding in their fields of research There seems to be a slight preponderance of authors from the Boston area but the editors have a broad outlook on research in the field and have enlisted contributors from all parts of the USA from Europe (including Scandinavia) and other continents The contributors in their turn have taken the same attitude and their lists of references are as a rule exhaustive and un biased

In this review it is only possible to give a short description of the contents The book is divided into two main parts the first entitled Mechanisms of bronchial asthma After an introduction to the history of asthma two excellent chapters follow with information and discussions that should serve as an invaluable base for understanding the nature of bronchial asthma and its place in the field of lung diseases J G Scadding London deals with definitions and clinical considerations G L Snider Boston writes on the interrelationship of asthma chronic bronchitis and emphysema It is highly desirable that everybody concerned with clinical research on chronic airflow obstruction should consider the clear views of these two authors on definitions and terminology After these introductory chapters there are descriptions of the epidemiology and genetical aspects

A separate section is devoted to a detailed description of mechanisms in asthma including fundamentals of immunology theories immediate hypersensitivity pharmacological mediators β adrenergic theory and blockade cholinergic mechanisms and late asthmatic reactions An introduction to immunology by G J Gleich and T B Thomasi Jr (Mayo Medical School) opens this section followed by chapters on immune responses There is for instance a chapter on immunoglobulin E written by Scandinavian authors (H Bennich S G O Johansson and H von Bahr Lindstrom) and one on non immediate asthmatic reactions by J Pepys London W E Brocklehurst London introduces in an excellent review pharmacodynamics and mechanisms of asthma followed by a series of chapters on the autonomic regulation of the bronchi the role of prostaglandins etc This section on mechanisms of asthma is perhaps the most stimulating part of the book Clinical physiology and pathology including defence mechanisms and experimental asthma have however been given ample scope in well written chapters as have ethiology and environmental considerations The latter section contains an introductory review by P van Arsdel Seattle There is an excellent chapter on house dusts and mites by K Aas Oslo one on mold fungi and bronchial asthma by A W Frankland London etc

The second part of the book consists of two sections on diagnostics and therapeutics in asthma As often happens in reference books the description in the clinical part is more conventional than in the preceding theoretical part. To a great extent the contents should be well known to physicians concerned with practical management of asthma The summaries of modern literature are however useful and most of the chapters provide stimulating reading

It should be evident from this review that Weiss and Segal have produced an excellent book of reference which should be available at all institutions with a research interest in asthma or with special responsibility for diagnosis and treatment of the disease

Gunnar Dahlstrom Uppsala Sweden

Acta Med Scand 202 149–152 1977

Complement Studies in Adipose Patients Treated with Intestinal Bypass

B Broch Møller J Jensen and I Lind Nielsen

From the Nephrological Department Hvidovre Hospital and Surgical Department I Kommunehospitalet Copenhagen Denmark

ABSTRACT Seventeen consecutive patients subjected to jejunoileostomy for obesity have been investigated for complement abnormalities and cryoglobulinaemia The study took place 1–9½ years after the operation A concomitant clinical examination revealed recurrent arthritis in 6 (30%) of the patients In 6 of the patients complement abnormalities were found as activation of the classical pathway in 3 and activation of the alternative pathway in another 3 could be suspected from immunochemical data One patient showed activation of both the classical and the alternative pathway Two of the patients with arthritic symptoms belonged to the group showing activation of the alternative pathway It is suggested that deficient inactivation of bacterial products from intestinal bacteria (lipopolysaccharides) have a role in the complement abnormalities found No patient exhibited the cryoprotein complexes found earlier in this type of patients

Jejunoileal bypass for morbid obesity has become an accepted form of treatment (2 5 9 10) The operative mortality is low (1–2%) and the weight reduction is often satisfactory (2) However this operative procedure may be followed by several complications at either an early (intractable diarrhoea electrolyte imbalances) or a late stage (hepatic insufficiency urinary calculi renal tubular acidosis and arthritis) The severity of the late complications has in some cases led to reoperation with elimination of the bypass and subsequent resolution of clinical symptoms has been observed (11 13) However the exact significance of the late complications is still unknown as is the pathogenesis

The most frequent complication is recurrent arthritis observed in 13–30% of the cases (2 11) Generally a tenosynovitis involving wrists and fingers is found but greater joints can be affected Rheumatoid factors or antinuclear antibodies are not found In a recent paper Wands et al (13) analysed 3 patients with severe arthritis following intestinal bypass They were able to demonstrate that activation of complement took place through both the classical and the alternative pathway in the patient s serum during bouts of arthritis Furthermore in connection with arthritic symptoms cryoprotein complexes were found These complexes consisted of immunoglobulins and complement components It was shown that the immunoglobulin had antibody activity against certain intestinal bacteria (e g E coli) Reoperation was necessary in one patient whose arthritic symptoms cleared and cryoprotein complexes could no longer be detected During resolution of symptoms in two other patients and in two controls without arthritis no cryoprotein complexes were found

STUDY BASE

The study population comprised 17 consecutive patients 16 females subjected to jejunoileostomy for morbid obesity since Oct 1966 Their mean age was 38 years (range 22–63) For all but one patient the operative procedure was the same An end to-end anastomosis was established between the distal aspect of the oral 36 cm of jejunum and the ileum 12 cm from the coecum The remaining 90% of the small bowel closed orally remained in situ (9) Biopsy specimens of the liver taken from 11 patients showed slight to moderate fatty infiltration in 8 However liver function was judged normal from measurements of albumin coagulation factors ASAT and alkaline phosphatase

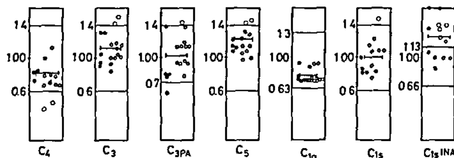


Fig 1 Complement values (arbitrary units) in sera from 17 patients following intestinal bypass for obesity ☐=normal limits (mean±2 S D) ●=patients with arthritic symptoms O=patients without arthritic symptoms

The examination for complement abnormalities took place 1-9½ years after the operation A concomitant clinical examination and history showed that 6 of the patients could be classified as having recurrent arthritis involving fingers with periodical swelling redness and morning stiffness These patients will be referred to as the group with arthritic symptoms Blood samples of 35 ml were taken at 10 a m in the supine position for analysis of plasma proteins (including complement components) antibodies against DNA antinuclear antibodies and rheumatoid factors Cryoproteins were investigated on another occasion (on the same day) as stated below *Plasma proteins* were quantified by a rocket immunoelectrophoresis using monospecific antisera (Behringwerke) as described earlier (7) *DNA antibodies* were determined by a modified Farr s technique (4) *Cryoproteins* were screened for on blood drawn at 37 C serum was isolated at the same temperature Analysis performed as described by Nakamura (8)

ontrol sera Control values for complement components were obtained from 20 healthy persons studied for 6 months as described in detail elsewhere (3) Normal values for complement ratios (C3/C4 C3/C3PA and C3PA/C4) were determined from these control individuals All control values given are mean±2 S D

RESULTS

Complement studies In general we found no significant changes in the mean values of the complement components in the total group of patients However we observed a tendency to low serum levels of C_{1q} and C4 (Fig 1) Furthermore a significant elevation of $C1_{INA}$ was documented especially in the patients with arthritic symptoms The mean values cannot be used to decide whether activation of complement is present and to answer this question we studied the ratio between certain complement components and compared these with the ratios of the control persons The ratios studied together with our interpretation are shown in Table I Fig 2 indicates that complement activation takes place in some of the patients Three patients showed activation of the classical pathway (C3/C4 ratio elevated) while another 3 showed activation of the alternative pathway (C3/C3PA ratio elevated) One of the patients with activation of the classical pathway displayed a normal C3PA/C4 ratio indicating a concomitant activation of the alternative pathway Arthritic symptoms were found in two of the patients showing activation of the alternative pathway while none of the patients showing activation of the classical pathway had arthritic symptoms

Immunoglobulins Three patients none of whom had arthritis had a slightly elevated serum level of

Table I *Normal values for complement ratios and the interpretation of elevated or low ratios*

	C3/C4	C3/C3PA	C3PA/C4
Normal values	0 80–1 76	0 74–1 42	0 59–1 89
Activation of classical pathway	High	Low	High
Activation of alternative pathway	Normal or low	High	Low

Table II *Certain plasma proteins (µmol/l) in 17 patients following intestinal bypass for obesity (mean±S D) and normal values*

	Patient values	Normal values
Albumin	512±60	510–740
α 1-glycoprotein	18±4.8	12 [illegible]
Haptoglobin	20 8±9 9	2–35
Fibrinogen	12 7±3 4	7 0–14.5
α 2 macroglobulin	4 4±1 25	1 3–4 1

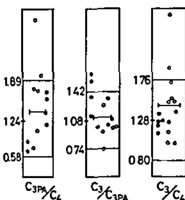


Fig 2 Complement ratios (arbitrary units) in 17 patients following intestinal bypass for obesity Symbols as in Fig 1

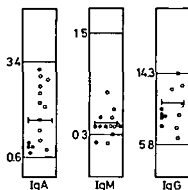


Fig 3 Immunoglobulin levels (g/100 ml) in sera from 17 patients following intestinal bypass for obesity Symbols as in Fig 1

IgG while IgA levels were normal IgM showed a tendency to be low but was generally within the normal limit (Fig 3)

Other plasma proteins Albumin was slightly decreased as shown in Table II Rather low levels were found in 7 patients (mean±S D 451±22 μmol/l) There was no correlation between low levels of albumin and complement abnormalities Acute phase reactants (α 1 glycoprotein and haptoglobin) were normal while fibrinogen was elevated in 4 patients (mean±S D 17 6±3 0 μmol/l) This was not correlated to low albumin levels or complement abnormalities The non specific protease inhibitor α 2 macroglobulin was substantially elevated in 10 patients (mean±S D 5 2±0 8 μmol/l) In 5 out of 6 patients showing complement activation α 2 macroglobulin was elevated indicating an inflammatory reaction

Antinuclear antibodies and rheumatoid factors Antinuclear antibodies were seen in 3 patients and rheumatoid factors in 1 patient There was no correlation between these findings and complement abnormalities or arthritis

DNA antibodies None of the patients showed elevated titres of DNA antibodies the observed mean value was 0 09±0 02 (S D)

Cryoproteins Except for insignificant cryofibrinogenaemia and one instance of IgG cryoglobulin (0 09 mg protein/ml) no cryoproteins were found

DISCUSSION

The finding of circulating cryoprotein complexes in patients with arthritis following intestinal bypass operations for morbid obesity and the coexisting complement activation (13) have important pathogenetic bearings on diseases such as rheumatoid arthritis and the arthritides in various intestinal diseases (ulcerative colitis Crohn s disease and Whipple s disease) As strongly indicated in the study by Wands et al (13) on only 3 patients with active arthritis intestinal bacteria play an important role in certain arthritic conditions It has been supposed that gramnegative bacteria or at least bacterial lipopolysaccharide can pass the intestinal epithelium (12) but in healthy persons the reticuloendothelial system in the liver is able to inactivate lipopolysaccharide (6) However when hepatic function is compromised as in some adipose patients (2) both before and after bypass procedures or when the load of lipopolysaccharide is heavy (6) the liver is unable to eliminate the toxic molecule and serum factors such as complement and certain esterases (1) are important in the clearance process When degradation is insufficient we suggest that circulating complexes supervene such as found by Wands et al (13) One might expect complement abnormalities to be present in the absence of overt symptoms if our hypothesis concerning intestinal bacteria in the pathogenesis of arthritis in these patients is correct

In agreement with this we found complement activation to be probable in at least 6 patients (30%) In 3 patients activation occurred through the classical pathway in 2 through the alternative pathway and in 1 patient through both these pathways Of the 3 patients showing activation through the alternative pathway a history ecu

arthritis was documented in 2 Complement in activator ($C1_{INA}$) was elevated in 11 patients, including 5 of the 6 with arthritis perhaps indicating an increased turnover of complement components not reflected in the ratios investigated A further argument could be the substantial elevation of the non specific protease inhibitor (α 2 macroglobulin) in 8 of these 11 patients

That intestinal bacteria are involved is also suggested in occasional reports of remission of arthritis in bypass operated patients following broad spectrum antibiotics (tetracycline) and the striking response of the symptoms in Whipple s disease following treatment with tetracycline

The high incidence of arthritis (30%) in the present patients may be explained by the long observation time (more than 9 years in some patients) and we would like to point out that only one of our patients had incapacitating arthritis for longer periods This patient also suffered from nephrocalcinosis and underwent a renal biopsy which did not show deposition of immune complexes in glomeruli In accordance with earlier reports on arthritis in such patients (11) we have not found rheumatoid factors or X ray changes compatible with rheumatoid arthritis except in one patient robably suffering from rheumatoid arthritis prior the operation None of our patients exhibited vated titers of DNA antibodies We were unable o find significant cryoprotein complexes (13) in any of our patients which can be explained by the fact that none of them had an active arthritis at the time of the investigation

REFERENCES

1 Back U Broch Møller B & Bøg Hansen T C Host resistance to lipopolysaccharides in the pathogenesis of multiple sclerosis and membranoproliferative glomerulonephritis Lancet 2 188 1976
2 Baden H & Quade F Ekstrem fedme behandlet med jejuno-ileal shunt operation Månedsskr Prakt Lægegern 54 61 1976
3 Broch Møller B Back U Raun N & Gad I Plasma proteins in healthy humans In preparation
4 Christiansen E Ullman S & Halberg P DNA antibodies in sera from patients with systemic lupus erythematosus Acta Derm Venereol 55 195 19 6
5 Fikri E & Casella R R Jejunoileal bypass for massive obesity Ann Surg 179 460 1974
6 Greisman S E & Hornick R B Mechanisms of endotoxin tolerance with special reference to man J Infect Dis (Suppl) 265 1973
7 Laurell C B Quantitative estimation of proteins by electrophoresis in antibody containing agarose gel In Prot Biol Fluids 14 (ed H Peeters) pp 499–50? Elsevier Amsterdam 1957
8 Nakamura R M Cryoglobulins and relationship to various diseases In Immunopathology—Clinical laboratory concepts and methods pp 184–195 Little Brown and Co Boston 1974
9 Schwartz H & Jensen H E Jejuno-ileostomy in the treatment of obesity Acta Chir Scand 139 551 1973
10 Scott H W Dean R Shull H J Abram H S & Webb W New considerations in use of jejunoileal bypass in patients with morbid obesity Ann Surg 177 723 1973
11 Shagrin J W Frame B & Duncan H Polyarthritis in obese patients with intestinal bypass Ann Intern Med 75 377 1971
12 Triger D R & Wright R Hyperglobulinaemia in liver disease Lancet I 1494 1973
13 Wands J R LaMont J T Mann E & Isselbacher K J Arthritis associated with intestinal bypass procedures for morbid obesity N Engl J Med 294 121 1976

Acta Med Scand 202 153–161 1977

Metabolism of Very Low Density Lipoprotein of S_f 100–400 in Type V Hyperlipoproteinaemia

Fiona C Ballantyne [1] David Ballantyne [2] Anders G Olsson
Stephan Rossner and Lars A Carlson

*From King Gustaf V Research Institute and the Department of Internal Medicine
Karolinska Hospital Stockholm Sweden*

ABSTRACT The metabolism of the apolipoproteins of very low density lipoprotein (VLDL) was studied in three subjects with primary type V hyperlipoproteinaemia (HLP) after injection of autologous ^{125}I labelled VLDL of S_f 100–400 The fractional disappearance rate of total radioactivity was stable during the two-week period of study Transfer of apolipoprotein C (apo-C) from VLDL to high density lipoprotein (HDL) occurred both in vivo and in vitro upon mixing the labelled VLDL preparation with serum and significant amounts of ^{125}I labelled apolipoprotein B (apo-B) and apo-C were recovered in the chylomicron fraction (S_f >400) in both situations The time courses of the disappearance of specific activity from VLDL (S_f 20–400) and its appearance in low density lipoprotein (LDL) are consistent with apo-B in VLDL (VLDL-B) being the precursor of that in LDL In comparison to studies in which the whole VLDL class (S_f 20–400) was labelled the rate of synthesis of VLDL B was slightly decreased and the fractional rates of catabolism (FCRs) of apo-B in VLDL and in the whole spectrum of VLDL+chylomicrons were markedly depressed In turn the rate of formation of LDL was low, and its FCR was probably slightly decreased The findings are consistent with the belief that in these three subjects the type V HLP is to a great extent due to impaired rates of formation and breakdown of apo-B in VLDL They do not exclude the possibility that the disorder is also caused by an increased rate of formation of chylomicrons

---

Present addresses
[1] University Department of Pathological Biochemistry Royal Infirmary Glasgow Scotland
[2] Division of Clinical Medicine Victoria Infirmary Glasgow Scotland

The lipoproteins of plasma have been classified into four main groups—chylomicrons VLDL LDL and HDL (1)—but it is becoming increasingly apparent that these have a dynamic interaction with both exchange and net transfer of lipid and protein The protein component of VLDL consists of several polypeptides including apo-B which forms 55% of the apolipoproteins in VLDL (17) and is identical to the major peptide (more than 95%) of LDL Most of the remaining apolipoproteins of VLDL are of the C type (11) three main groups of which have been described (17) VLDL is heterogeneous and can be separated into subfractions by different techniques By sequential ultracentrifugation through a density gradient three subfractions of S_f 100–400 60–100 and 20–60 can be obtained (18) Each VLDL subclass represents a continuous spectrum of lipoproteins in terms of size and density According to current concepts the larger VLDL particles are catabolized to the smaller ones and the ratios of protein to lipid and of apo-B to apo C increase during this process (9 14)

In vivo studies using radioiodinated VLDL (2 9) have indicated that there is rapid transfer of apo-C between VLDL and HDL and passage of apo-B through VLDLs to LDL More recently (25) the interrelationship between the B peptide in VLDL and LDL has been quantitatively studied in normal subjects and subjects with hyperlipoproteinaemias The findings support the concept that most if not all VLDL B is converted into LDL B and at least in subjects with normal triglyceride concentrations most if not all LDL B is derived from VLDL B No assessment was made of the metaboli

Table I *Clinical data*

Subj no	Sex	Age (y)	Weight (kg)	Mean concentration in plasma (mmol/l) Total Chol	Total Tg	VLDL+chylos Chol	VLDL+chylos Tg	LDL Chol	LDL Tg	HDL Chol	HDL Tg
1	♂	53	76 3	19 2	68 7	17 9	67 2	0 93	0 93	0 47	0 53
2	♂	46	94 8	12 2	30 6	10 1	29 5	1 76	0 66	0 54	0 43
3	♀	57	74 5	15 5	80 6	14 2	79 3	0 85	0 73	0 39	0 54
Mean		52	81 9	15 6	60 0	14 1	58 7	1 18	0 77	0 47	0 50
Upper normal limit											
Male				7 70	2 5	0 83	1 80	5 43	0 69	1 78	0 3[illegible]
Female				7 75	2 0	0 85	1 05	5 63	0 69	2 40	0 3[illegible]
Lower normal limit*											
Male				–	–	–	–	3 0	–	1 03	–
Female				–	–	–	–	3 1	–	1 37	–

* From Carlson and Ericsson (7) approximate values

In the present study the metabolism of VLDL has been studied in three subjects with severe primary type V hyperlipoproteinaemia This is a rare condition characterized in a fasting subject by the presence in plasma of chylomicrons (S_f >400) and of a marked increase in the cholesterol and triglyceride concentrations of VLDL of S_f 20–400 concentrations of LDL and HDL-cholesterol are variably low It is likely that these abnormal concentrations are due to disorders in the metabolism of chylomicrons and probably also of VLDL It was decided to study the metabolism of ^{125}I labelled "large" VLDLs of S_f 100–400 in order to follow the conversion of these to smaller VLDL particles in addition to assessing the exchange of apo-C with HDL and the transfer of apo-B to LDL

SUBJECTS

Each subject had been referred to the Lipid Clinic Karolinska Hospital and was characterized as having primary type V HLP by lipoprotein analyses on at least three occasions during the previous six months Secondary causes of type V HLP e g diabetes or alcoholism were excluded by clinical history and appropriate laboratory tests None had undergone therapy during the preceding three months The subjects were admitted to the Lipid Metabolism Unit where the procedures were fully explained to them and their informed consent was obtained A brief summary of their routine lipid analyses during the period of study is given in Table I "Normal" values (7) are given for comparison

METHODS

Preparation of ^{125}I labelled VLDL (S_f 100–400) Isolation Autologous VLDL (S_f 100–400) was used in each study After a 14-hour fast 50 ml blood was removed under sterile conditions from a cubital vein and collected into Li EDTA (1 3 mM final) All reagents were prepared under sterile conditions and equipment required for the preparation was sterilized before use Plasma was separated after low speed centrifugation To remove chylomicrons (12) plasma was overlayered with NaCl (0 15 M) and centrifuged in a Beckman model L5-75 ultracentrifuge using a 40 3 fixed angle rotor at 19000 rpm for 30 min ($7 8\times10^5$ g min) After removal of chylomicrons by tube slicing the plasma density was altered to d=1 065 kg/l by addition of solid NaCl and VLDL (S_f 100–400) was isolated from the chylomicron free plasma by centrifugation through a NaCl gradient at 35000 rpm for 144 min ("up to speed" time) in the L5 75 ultracentrifuge using a Beckman SW-40Ti 6 place swinging bucket rotor for 21 22×10^6 g min The procedure has been fully described (18) using a SW-41Ti rotor, the slightly longer tubes used with the SW-40Ti rotor were fitted with epoxy inserts to give a path length identical to that documented (18) 2 ml of plasma were placed in each tube and less than 0 5 ml VLDL (S_f 100–400) was removed from the top of each tube (total approximately 2 5 ml) after centrifugation 0 5 ml was retained for assessments of purity

Iodination The apolipoproteins of the VLDL (S_f 100–400) were radioiodinated with ^{125}iodine at pH 10 0 by a modification (16) of the iodine monochloride method (21) The amount of ICl added was such that less than one atom of iodine was introduced per particle of VLDL assuming a molecular weight of 260000–300000 for the whole protein complex in a VLDL particle of weight approximately 10^7 The efficiency of iodination was 10–20% The preparation was dialysed against 5 changes of 0 15 M NaCl/1 mM EDTA for 16 hours

Assessments For both the labelled and unlabelled lipoprotein preparations electrophoresis in agarose gel (22) with staining of lipid by Sudan Black indicated that only material of pre β mobility was present staining for protein by Coomassie Blue showed no contamination with other serum proteins Immunodiffusion (which does not exclude the presence of LDL) and two-dimensional

immunoelectrophoresis (electrophoresis in agarose followed by electrophoresis at right angles into agarose containing anti whole human serum) also supported the conclusion that the preparation contained only VLDL (pre β lipoprotein)

The final ¹²⁵I labelled preparation contained less than 5% free ¹²⁵I as determined after addition of carrier albumin by precipitation with trichloracetic acid (50 g/l final). To determine the amount of label bound to lipid an aliquot of the preparation was extracted with chloroform methanol (6). The chloroform phase was evaporated to dryness in a counting vial and the contents were resuspended in 0 15 M NaCl before counting. The proportion of ¹²⁵I bound to lipid ranged from 6 to 10%

Both the B and C apolipoproteins of VLDL became labelled during the iodination procedure and approximately 50% of the label was bound to B peptide as determined after precipitation of apo-B from VLDL by tetramethylurea (TMU) (13 14) for all three preparations and also by separation of one preparation of delipidated VLDL on G 150 Sephadex (4). The flotation on ultracentrifugation of the ¹²⁵I labelled VLDL (S 100–400) was assessed on aliquots of the final preparation to which had been added carrier albumin. All the radioactivity was recovered in the top fraction (VLDL) after ultracentrifugation at d 1 006 kg/l (5). Density gradient assessment (18) of the preparation showed that more than 80% of the activity was recovered in the first VLDL fraction (S 100–400) with small amounts of activity in the second (S 60–100). However when the preparation was mixed in vitro with the patient s unlabelled plasma about 15–30% of activity was recovered in the chylomicron fraction on ultracentrifugation (presumably at least partly due to exchange of C peptides). When the labelled preparation was added to chylomicron free plasma about 60% of activity was recovered in the VLDL fraction of S 100–400 with the remaining activity in the smaller VLDL particles and in HDL (due to exchange of C peptides)

The final preparation of ¹²⁵I labelled VLDL (S 100–400) was diluted with sterile human serum albumin (Kabi) at a final concentration of 30–40 g/l. It was passed through a sterile filter of pore size 0 45 μm (Millipore) before injection. A maximum of 24 hours elapsed between removal of the initial blood sample and reinjection of the labelled VLDL

Study protocol

The subjects received a normal ward diet their body weights remained constant throughout the study. They took 200 mg potassium iodine (¹²⁷I) orally in divided doses for 4 days before and throughout the study to minimize uptake of radioiodine by the thyroid. The sterile ¹²⁵I labelled VLDL (S 100–400) was injected i v after an overnight fast. The contents of the syringe were weighed and after injection the residue was washed to a suitable volume with human serum albumin (approximately 30 g/l final Kabi) and the ¹²⁵iodine was measured to determine the exact doses administered. A portion of the ¹²⁵I labelled VLDL was retained to act as a standard. In subject 2 blood specimens were taken 10 min after injection to allow determination of plasma volume hourly for the next 8 hours and then daily at 08 00 (fasting) for 2 weeks whereas in the other two subjects blood samples (20 ml) were removed at 10 min 2 hours 6 hours and daily (fasting) thereafter for 2 weeks. Blood was collected into Li EDTA (final 1 3 mM). Collections of urine in 24-hour aliquots (20 00–20 00) were made throughout this period

Sample analysis

To separate chylomicrons 4 ml plasma was placed in an 1 27×6 35 cm Beckman cellulose nitrate tube overlayered with NaCl/EDTA of d=1 006 kg/l (5) and centrifuged in a 40 3 rotor as described above. The tube was then sliced the chylomicron fraction (top) made to 3 ml and the chylomicron free plasma (bottom) made to 5 ml both with the solution of d=1 006 kg/l. All chylomicron free plasmas were then subjected to sequential preparative ultracentrifugation (5) first at plasma density (d 1 006 kg/l) then at d 1 063 kg/l to separate the conventional lipoprotein classes of VLDL LDL and HDL. On three occasions during each study VLDL was also separated into three fractions S 100–400 60–100 and 20–60 in the SW40Ti rotor (18). From all samples of chylomicrons total VLDL and VLDL subfractions B and C apolipoproteins were separated by a modification of the TMU precipitation technique (13 14). The TMU soluble peptides (apo-Cs but including arginine rich peptide) were aspirated and then precipitated and delipidated with 1 1 chloroform methanol (Carlson unpublished). This also served to remove TMU which has been said to interfere with the Lowry reaction (19) and the C peptides were redissolved in NaOH

The ¹²⁵I activities of standards (dilutions of dose) total plasma (with and without removal of chylomicrons) urine and all individual lipoprotein and apolipoprotein fractions were measured in an automatic single channel spectrometer (Packard). Correction was made for quenching of ¹²⁵I at high salt concentrations (9). After delipidation as described above protein concentrations were estimated (19) on chylomicrons and after removal of chylomicrons on total plasma LDL total VLDL and VLDL subfractions the protein concentrations of the B and C components of chylomicrons and VLDLs (apo-B total apo-C) were also measured. No correction was considered necessary for the traces of apo-A known to occur in VLDL and chylomicrons (17). Estimation of the total protein concentration of all plasma samples allowed correction for changes in plasma volume during the studies and for variations in technique when removing chylomicrons from samples

From blood samples on 4 days during the study whole plasma as well as the corresponding isolated lipoprotein fractions were extracted manually with isopropanol to allow simultaneous estimation of routine lipids with an AutoAnalyzer I (Technicon). Cholesterol was estimated photometrically by Technicon method N24a (3) and triglyceride by a fluorometric procedure (Technicon method N78) (15). Urinary creatinine content was also estimated by Technicon methodology (method N11B) and urine volumes corresponding to mean creatinine content were calculated to correct for errors in collection of urine

Data analysis

¹²⁵Iodine data have been expressed both as fractions of the dose of radioactivity injected and also as specific

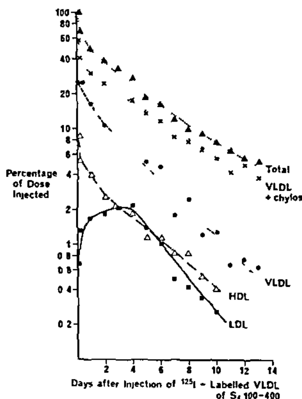


Fig 1 Activity in the plasma lipoproteins of subject 1 ▲=total plasma (including chylomicrons) ×=VLDL +chylomicrons (S_f >20 d<1 006) ●=VLDL (S_f 20–400 d<1 006 after removal of chylomicrons) ■=LDL (1 006–1 063) △=HDL (d>1 063)

ities Radioactivity (expressed as fraction of dose and specific activity)/time curves for total plasma chylomicrons VLDL and HDL were fitted to mono or biexponential functions as appropriate by programmes constructed for a modified Alpha LSI computer (Computer Automation Inc) and a Wang 700 electronic calculator (Wang Europe Ltd) LDL activity with time rose to a maximum and its subsequent disappearance fitted best to a monoexponential function The specific activity/time curves for the disappearance of apo-B from VLDL and of its appearance in LDL were compared to establish whether there was a precursor–product relationship between the two It has been shown (30) that a necessary prerequisite of such a relationship is that the two specific activity curves should intersect at the maximum of the product curve the product curve thereafter lying above the curve for the immediate precursor This aspect of precursor–product relationships between apo-B in VLDL and LDL has been discussed more fully by Sigurdsson et al (25)

The subjects could be assumed to be in steady state and therefore the fractional catabolic rate (FCR=fraction of the plasma pool catabolized per unit of time) could be calculated for monoexponential functions such as the disappearance of LDL activity from the slope of the exponential by the formula FCR=0 693/$t_{\frac{1}{2}}$ When the disappearance of activity was a biexponential function e g disappearance of total activity the equations derived from the models of Matthews (20) were applied using programmes constructed for the Alpha LSI computer and Wang 700 calculator Fuller descriptions of the equations are available (16 20) Total disappearance rates were also calculated from the daily urinary clearance of activity

Interpretation of the present data is more complex than in the situation of e g LDL studied with ^{125}I labelled LDL where there is no transfer of label to other lipoproteins and where only one apolipoprotein (apo-B) is present For example the present study is complicated by the fact that ^{125}I labelled apo-C was transferred from VLDL to HDL with probably subsequent exchange of labelled apo-C between the two lipoprotein classes (2 8 9) In this case a value derived by Matthew s equation for the fractional disappearance rate (FDR=fraction of the plasma pool leaving plasma per unit of time) of apo-C from HDL cannot be equated with that for the FCR of the apo-C A further complication is that LDL metabolism has been characterized from the disappearance of ^{125}I transferred to LDL from VLDL B whereas it would have been theoretically more correct to have made an independent assessment of LDL e g by simultaneous injection of ^{131}I labelled LDL However as discussed later the findings of Sigurdsson et al (25) lend support to the present approach

RESULTS

Disappearance and redistribution of total radioactivity

In each of the three subjects the clearance of total radioactivity (urine ^{125}I/plasma ^{125}I U/P) remained constant throughout the period of study The absence of excessive initial excretion of ^{125}I serves as a check on the quality of the preparation However it is surprising that the total U/P ratio did not vary significantly during the study since the catabolism of the VLDL of S_f 100–400 (10% label in lipid and the rest distributed equally between apo B and apo-Cs) is a complex process

Patient 2 from whom blood samples were collected hourly for the first 8 hours after injection showed little change in ^{125}I in total plasma or in VLDL during this period Thereafter in *all three* subjects as exemplified for subject 1 in Fig 1 the disappearance of total radioactivity from plasma fitted well (r>0 995) to a biexponential function and could therefore be analysed by the method of Matthews (20) The FDRs of total plasma B+C apolipoproteins derived by this method were 0 31 0 35 and 0 33 days^{-1} (mean 0 33) These values (although consistently slightly higher) were in good agreement with those for FDRs calculated from the daily U/P ratios (mean±S D 0 27±0 05 0 31±0 05

Table II *Parameters for metabolism of apo-B*

Subj no	Apo-B in VLDL+ chylomicrons $t_{\frac{1}{2}}$ (d)	FCR (d^{-1})	Apo-B in VLDL Conc (mg/l)	$t_{\frac{1}{2}}$ (d)	FCR (d^{-1})	Synthetic rate (mg/kg/d)	Apo-B as LDL Conc (mg/l)	$t_{\frac{1}{2}}$ (d)	FCR (d^{-1})	Synthetic rate (mg/kg/d)
1	3 01	0 471*	500	2 62	0 4.9	8 14	650	2 28	0 304	7 43
2	0 88	0 788	470	1 01	0 686	12 1	638	2 42	0 286	6 85
3	2 89	0 240	530	1 36	0 510	12 0	588	2 17	0 319	8 34
Mean	2 26	0 500	500	1 66	0 542	10 7	625	2 29	0 303	7 54

*Biexponential see Methods

and 0 26±0 03 respectively) Within the VLDL and chylomicron classes most of the [125]I was initially found in the large VLDL particles (S_f 100–400) but a substantial proportion (15–46%) was also present in the arbitrarily-defined chylomicron class With time activity passed from these larger particles into the subfractions containing the smaller VLDL particles (S_f 20–60 and 60–100)

After injection of the preparation there was a transfer of [125]I to HDL At 10 min between 5 and 9 5% of the total activity injected was detected in HDL (Fig 1) It was assumed that much of this activity was in apo-C no estimate was made of the proportion of [125]I labelled lipid (initially 6–10%) in VLDL which transferred to HDL A similar rapid exchange was also found in vitro HDL metabolism is discussed further below in relation to the exchange of C peptides between VLDL and HDL

Satisfactory activities were obtained for total [125]I and for VLDL and chylomicrons throughout the study However in the last 3 days activities in HDL and LDL could not be resolved accurately from background and therefore have not been used

Transfer of apo-B from VLDL to LDL

No normal ranges are at present available for the concentration of apo-B in VLDL and LDL for the Stockholm population but based on experience of analyses of normal and hyperlipidaemic subjects the VLDL B concentrations of the three subjects with type V HLP were clearly increased whereas their LDL protein concentrations were slightly decreased (Table II) Radioactivity in LDL was initially very low (less than 1%) but rose to a maximum (2 2–3 5% of dose injected approximately 10% of remaining activity) approximately 3 days after injection Thereafter the disappearance of the [125]I labelled LDL was monoexponential (Figs 1 and 2) As will be discussed this is in contrast to the biexponential disappearance which is obtained after i v injection of radioiodinated LDL

Specific activity/time curves were plotted for LDL and VLDL (S_f 20–400) to determine if a precursor–product relation existed between them As shown in the example in Fig 2 the specific activity/time curves of apo B in VLDL (S_f 20–400) and of LDL intersected at the maximum of the LDL curve and thereafter the VLDL B specific activity was never greater than that of LDL These findings are consistent with the precursor–product relationship believed to exist between them (25, 30) Rates

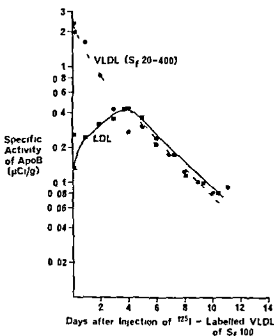


Fig 2 Specific activity of apo-B in plasma L of subject 1 Symbols as in Fig 1

Table III *Parameters for metabolism of apo C*

	Apo C in VLDL+ chylomicrons		Apo-C in VLDL				Apo C in HDL[a]	
Subj no	$t_{½}$ (d)	FDR (d ⁻¹)	Conc (mg/l)	$t_{½}$ (d)	FDR (d ⁻¹)	ADR (mg/kg/d)	$t_{½}$ (d)	FDR (d ⁻¹)
1	5 21	0 192[a]	430	4 92	0 199[a]	3 25	3 00	0 423[a]
2	3 44	0 201	420	2 60	0 267	4 22	3 36	0 [illegible][a]
3	4 52	0 153	410	4 31	0 161	2 93	3 32	0 360[a]
Mean	4 39	0 182	420	3 94	0 209	3 47	3 23	0 338

Parameters calculated from total radioactivity [a] Biexponential see Methods

of catabolism and synthesis of apo B in the lipoprotein classes are given in Table II These values were calculated from the decrease in apo-B specific activities Similar values were obtained when the disappearance of apo B total activities from each lipoprotein class were analysed which gives further confirmation that the subjects were in steady state Subject 2 who was the least severely affected showed the fastest fractional rate of catabolism of apo B both from VLDL and the spectrum of VLDL+chylomicrons Similar values were derived for the FCR of LDL in each subject The absolute rate of synthesis of apo B in VLDL was consistently higher than that of LDL (metabolized from VLDL)

insfer of apo C between VLDL and HDL

ce the apo C concentration in HDL was not easured specific activities in HDL could not be calculated The concentration of apo C in VLDL was increased (see comments above with respect to apo B concentration) but the concentration in chylomicrons although increased cannot be accurately quoted Parameters characterizing the disappearance of apo C from plasma are given in Table III Due to the exchange of C peptides between VLDL and HDL the disappearance rates for apo C cannot be equated with catabolic rates The results for apo C in VLDL were more similar in the two subjects with marked chylomicronaemia than in the subject with less severe type V HLP In these two subjects the FDR of apo-C from HDL was much faster than that from VLDL and VLDL+chylomicrons whereas in the third subject the rates were similar in each lipoprotein class

DISCUSSION

The three subjects investigated had severe type V HLP In addition to marked increases in cholesterol and triglyceride in chylomicrons the whole VLDL class and VLDL subfractions they had increased apo B and apo C concentrations within these lipoproteins This is in agreement with studies showing that an increase in the concentration of protein in VLDL is a feature of hypertriglyceridaemia (23) and that the concentration of apo B in VLDL measured by radioimmunoassay is increased in types IV and V HLP (24 28)

Analyses of total ^{125}iodine radioactivities show that each subject catabolized about 30%/day (calculated either from U/P activities or from plasma disappearance alone) of the total plasma pool of ^{125}I labelled lipoproteins This proportion remained constant during the period of study but it must be a complex function since in the injected material about 90% of the activity was distributed equally between apo B and the apo-Cs with about 10% of the activity in lipid and after injection there was redistribution of activity Apo C was rapidly transferred to HDL with presumably subsequent exchange of labelled apo-C with VLDL (8) apo-B passed more slowly through the VLDL class before being converted to LDL It was unexpected that after injection the clearance of total activity was similar in the first few days to the values obtained later in the study since initially little of the excreted activity could be ascribed to apo-B catabolized via LDL or to apo-C catabolized at least partly via HDL Some of the apo Cs may be able to be broken down directly from VLDL but the data do not provide convincing evidence that apo-B can be catabolized from VLDL without first passing through LDL

Although VLDL (S_f 100–400) was used in this study a significant proportion of activity was recovered in the chylomicon fraction (S_f >400) This may be partly due to exchange of apo-C between VLDL and chylomicrons Further the distinction

een chylomicrons and large VLDL particles somewhat arbitrary one and aggregation of)Ls may have occurred both in vivo and also centrifugation since the same findings were ined with in vitro assessments These com ts do not exclude the possibility of some re ing of apo-B from smaller VLDLs or LDL to omicrons In this context is is of interest that ur studies of the metabolism of [125]I labelled (Ballantyne et al in preparation) no activity recovered in other lipoprotein fractions in ects with types II or IV HLP whereas in one ne present subjects (no 3) with type V HLP ificant amounts of [1] I were recovered in the omicron fraction after injection of [125]I labelled This was not a simple in vitro effect but it not possible to resolve whether the chylomi ns were able to accept free [12] iodine liberated r breakdown of LDL or to take up whole [125]I lled LDL *molecules or to accept B protein from* L

n the present study the LDL activity was ini y very low which is consistent with the concept no rapid transfer (analogous to the transfer of -C from VLDL to HDL) occurs between apo-B rge VLDL particles or chylomicrons and LDL L was estimated as the lipoprotein of density)6–1 063 to include all B peptide within the LDL s It has been shown (17) that the lipoprotein in density range 1 006–1 019 bears more resem ce to VLDL than to LDL However in a study uding two subjects with type IV HLP and one h type V HLP (25) the concentration of total protein in the density class 1 006–1 019 kg/l was nall proportion of that in the whole LDL density ge

The specific activity/time curves of apo-B in DL (S_f 20–400) and of LDL intersected at the ximum of the LDL curve and thereafter the LDL ve lay above the VLDL B curve This is consis t with apo-B in this VLDL class being the pre sor of LDL in these three subjects with type V P It should be re emphasized that intersection specific activity curves is compatible with but es not prove a precursor–product relationship) After intersection the LDL specific activity ve was only slightly higher than that of the DL (S_f 20–400) curve Perhaps a more significant ation would have been found particularly after ection of VLDL of S_f 20–60 if LDL had been mpared with VLDL of S_f 20–60 since this may be a more immediate precursor However insufficient analyses were made on this class of small VLDL particles to permit such a comparison From the disappearance of radioiodinated VLDL of S_f 20–400 in seven normal and hyperlipidaemic subjects (in cluding one with type V HLP) Sigurdsson et al (25) obtained results which also indicated that apo-B in VLDL is the precursor of LDL

It is of interest that the disappearance of LDL was monoexponential in all of our three subjects after the LDL specific activity/time curve reached its maximum in contrast to the two- or three expo-nential disappearance function obtained after i v injection of radioiodinated LDL (16 26 28) VLDL B is catabolized at various ill defined sites and after injection of [125]I labelled VLDL B there would be a continuing process of VLDL B break down and therefore at least initially a net passage of [1] I labelled LDL into plasma After i v injection of [1] I labelled LDL the initial phase in plasma re flects not only catabolism of LDL but also to a greater extent net passage of [1 5]I labelled LDL out of plasma to extravascular sites until after equili bration a second or even third plasma exponential is found which reflects mainly but not entirely catabolism (depending on relative intra and extra vascular specific activities) This has relevance to the studies of Steinberg (29) which have indicated that there is a large extravascular pool of LDL in the liver After i v injection of [1 5]I labelled VLDL (S_f 100–400) this pool would presumably have freely equilibrated with the [1 5]I labelled LDL (derived from catabolism of small VLDL particles S_f 20–60) before or at least at about the same time as this LDL was transferred into plasma

Although the present study indicates that VLDL B is the precursor of LDL the quantitative results suggest that the rate of VLDL B synthesis is higher than that of LDL synthesis This may indi cate that not all VLDL B is converted to LDL A similar difference was found (25) in four of eight subjects with normal plasma triglyceride concentra tions However as discussed by these authors one cannot exclude the possibility that the dis crepancies between results for VLDL B and LDL synthetic rates arise at least partly from purely technical factors For example it would have been theoretically more correct to calculate parameters of LDL metabolism from the disappearance of [131]I labelled LDL injected at the same time as the [125]I labelled VLDL although in a study of 7 subjects

including 2 with HLP but none with elevated triglyceride concentrations (25) almost identical disappearance rates for LDL were found after simultaneous injection of VLDL and LDL, labelled with different iodine isotopes

When the present study was undertaken the decision was made to study VLDL metabolism by injection of [125]I labelled VLDL subfraction of S_f 100–400 in order to have a clearly defined material and to be able to follow its passage into smaller VLDL particles No normal subjects have been investigated and it would be difficult to isolate sufficient normal material to reinject autologous VLDL of S_f 100–400 within 24 hours of removal of blood for the preparation However a few studies are available in which radioiodide labelled whole VLDL (S_f 20–400) was injected for example the small series of Eisenberg et al (2 9) and the larger series of Sigurdsson et al (25 27) which included eight subjects with type IV HLP four with primary type V and seven with secondary type V HLP (27) The values derived for the fractional catabolic rate of VLDL B are markedly lower in the present subjects being only about 10% of the rates for eight subjects with normal lipids and also lower than in subjects with types IV and V HLP (27) The rate of synthesis of VLDL B in the present group was probably decreased in contrast to the increased [s]ynthetic rate in the study of hypertriglyceride[m]ic subjects (27) However it must be re[e]mphasized that the present study and the above studies (25 27) are not strictly comparable Another possible source of difference is that our three subjects had very severe type V HLP (Table I)

Published normal values for LDL catabolic rates vary (16 25 26 28) at least partly due to differences in injected material diet etc The values for the FCR of LDL in the present three subjects with type V HLP were lower than in the normal series (16 26 28) and the values for LDL synthetic rate were lower than in the published normal series This contrasts with the reports that in type V HLP the FCR of LDL is increased in the presence of a decreased (28) or normal (26) absolute turnover rate

The results of the present study for apo-C radioactivity are difficult to interpret because of the rapid transfer both in vivo and in vitro of apo-C from VLDL to HDL and a presumed subsequent exchange (8) between the two classes It cannot therefore be assumed that disappearance rates accurately reflect catabolism The FDR of apo-C from HDL was much higher than from VLDL in the two subjects with severe chylomicronaemia but it probably cannot be assumed that HDL is the main vehicle for the catabolism of apo C and the findings may be influenced by the fact that the smaller HDL molecule can leave the plasma compartment with greater facility than the much larger VLDL particles It is difficult to compare these findings with those (2 9) from a small series of subjects (including one with type V HLP) because of differences in injected material and because the present study was continued for a much longer period At least initially the disappearance curves of apo C from VLDL and HDL (after transfer from VLDL) in the three subjects with type V HLP were qualitatively similar to those found in normal subjects However in the normal subjects (2 9) [125]I labelled apo-C disappeared from VLDL and HDL with half lives of less than one day whereas disappearance rates were significantly slower from both lipoprotein classes in the present series It therefore seems reasonable to conclude that as with VLDL B the fractional rate of apo C catabolism was also depressed

These three subjects with severe type V HLP had markedly increased concentrations of cholesterol triglyceride and apo B and apo-C within the whole VLDL class It is reasonable to conclude that the metabolism of VLDL was qualitatively similar to that in normal subjects and in those with other hyperlipoproteinaemias since they were able to convert large VLDL particles (S_f 100–400) to smaller VLDL particles to convert apo B from VLDL to LDL and to exchange apo-C with HDL The rate of formation of VLDL B was probably slower than normal The FCR of VLDL C was probably decreased and the FCR of VLDL B was markedly depressed In turn the rate of formation of LDL (which can be regarded as a final breakdown product of VLDL) was decreased with probably a slightly lowered fractional rate of LDL catabolism These defects would lead to an accumulation of large VLDLs of S_f 100–400 and chylomicrons However it is possible that there was also excessive formation of chylomicrons

ACKNOWLEDGEMENTS

This study was supported by grants from the Swedish Medical Research Council (19X 204) and the Swedish National Association against Heart and Chest Diseases

REFERENCES

1 Beaumont J L Carlson L A Cooper G R Fejfar Z Fredrickson D S & Strasser T Classification of hyperlipidaemias and hyperlipoproteinaemias Bull WHO 43 891 1970
2 Bilheimer D W Eisenberg S & Levy R I The metabolism of very low density lipoprotein proteins I Preliminary in vitro and in vivo observations Biochim Biophys Acta 260 212 1972
3 Block W D Jarrett K J & Levine J B In Automation in analytical chemistry (ed L T Skeggs) pp 345–347 Mediad New York 1965
4 Brown W V Levy R I & Fredrickson D S Further separation of the apoproteins of the human plasma very low density lipoproteins Biochim Biophys Acta 200 573 1970
5 Carlson K Lipoprotein fractionation J Clin Path 26 (Suppl) 5 32 1973
6 Carlson L A Determination of serum triglycerides J Atheroscler Res 3 334 1963
7 Carlson *L A & Ericsson M Quantitative and* qualitative serum lipoprotein analysis Part 1 Studies in healthy men and women Atherosclerosis 21 417 1975
8 Eisenberg S Bilheimer D W & Levy R I The metabolism of very low density lipoprotein proteins II Studies on the transfer of apoproteins between plasma lipoproteins Biochim Biophys Acta 280 94 1972
9 Eisenberg S Bilheimer D W Levy R I & Lindgren F T On the metabolic conversion of human plasma very low density lipoprotein to low density lipoprotein Biochim Biophys Acta 326 361 1973
10 Eisenberg S Stein O & Stein Y Radioiodinated lipoproteins absorption of ^{125}I radioactivity by high density solutions J Lipid Res 16 468 1975
11 Gustafson A Alaupovic P & Furman R H Studies of the composition and structure of serum lipoproteins Physico-chemical characterisation of phospholipid protein residues obtained from very low-density human serum lipoproteins Biochim Biophys Acta 84 767 1964
12 Hatch F T & Lees R S Practical methods for plasma *lipoprotein analysis Adv Lipid Res* 6 2 1968
13 Kane J P A rapid electrophoretic technique for identification of subunit species of apoproteins in serum lipoproteins Anal Biochem 53 350 1973
14 Kane J P Sata T Hamilton R L & Havel R J Apoprotein composition of very low density lipoproteins of human serum J Clin Invest 56 1622 1975
15 Kessler G & Lederer H In Automation in analytical chemistry (ed L T Skeggs) pp 341–344 Mediad New York 1965
16 Langer T Strober W & Levy R I The metabolism of low density lipoprotein in familial type II hyperlipoproteinemia J Clin Invest 51 1528 1972
17 Lee D M & Alaupovic P Composition and concentration of apolipoproteins in very low and low density lipoproteins of normal human plasma Atherosclerosis 19 501 1974
18 Lindgren F T Jensen L C & Hatch F T In Blood lipids and lipoproteins (ed G J Nelson) pp 181–274 Wiley New York 1972
19 Lowry O H Rosebrough N J Farr A L & Randall R J Protein measurement with the Folin phenol reagent J Biol Chem 193 265 1951
20 Matthews C M E The theory of tracer experiments with ^{131}I labelled plasma proteins Phys Med Biol 2 36 1957
21 McFarlane A S In Mammalian protein metabolism (ed H N Munro and J B Allison) vol I pp 297–341 Academic Press New York 1964
22 Noble R P Electrophoretic separation of plasma lipoproteins in agarose gel J Lipid Res 9 693 1968
23 Ruderman N B Jones A L Krauss R M & Shafrir E A biochemical and morphologic study of very low density lipoproteins in carbohydrate induced hypertriglyceridemia J Clin Invest 50 1355 1971
24 Schonfeld G Lees R S George P K & Pfleger B Assay of total plasma apolipoprotein B concentration in human subjects J Clin Invest 53 1458 1974
25 Sigurdsson G Nicoll A & Lewis B Conversion of very low density lipoprotein to low density lipoprotein A metabolic study of apolipoprotein B kinetics in human subjects J Clin Invest 56 1481 1975
26 — The metabolism of low density lipoprotein in endogenous hypertriglyceridaemia Eur J Clin Invest 6 151 1976
27 — Metabolism of very low density lipoproteins in hyperlipidaemia Studies of apolipoprotein B kinetics in man Eur J Clin Invest 6 167 1976
28 Simons L A & Williams P F The biochemical composition and metabolism of lipoproteins in type V hyperlipoproteinaemia Clin Chim Acta 61 341 1975
29 Steinberg D In Atherosclerosis III (ed G Schettler and A Weizel) pp 658–671 Springer Verlag Berlin 1974
30 Zilversmit D B The design and analysis of isotope experiments Am J Med 29 832 1960

Professors of Internal Medicine in Scandinavia in 1977

Denmark

University of Copenhagen

Gunnar Bendixen Rigshospitalet esp Immunology
Laurids Korsgaard Christensen Amtssygehuset i Herlev esp Endocrinology
Viggo Faber Blegdamshospitalet esp Infect Dis
Anders Tybjærg Hansen Rigshospitalet esp Cardiology
Jørn Hess Thaysen Rigshospitalet esp Nephrology
Sven Aage Killmann Rigshospitalet esp Haematology
Ib Lorenzen Hvidovre Hospital esp Rheumatology
Flemming Quaade Hvidovre Hospital esp Metabolic Diseases
Povl Riis Amtssygehuset i Herlev esp Gastroenterology
Niels Tygstrup Rigshospitalet esp Hepatology

University of Århus

Poul Bastrup-Madsen Århus Amtssygehus esp Hematology
Henning Gøtzsche Kommunehospitalet esp Cardiology
Knud Lundbæk Kommunehospitalet esp Diabetic Diseases
Villy Posborg Petersen Kommunehospitalet esp Nephrology

University of Odense

Bent Harvald Odense Sygehus esp Genetic Disease
Ejvind Kemp Odense Sygehus esp Nephrology

Finland

University of Helsinki

Pentti I Halonen University Central Hospital
Tatu Miettinen University Central Hospital
Esko A Nikkilä University Central Hospital
Otto Wegelius University Central Hospital

University of Turku

Lennart Kalliomaki University Central Hospital

University of Oulu

Wille Kaipainen University Central Hospital

University of Kuopio

Kalevi Pyorälä University Central Hospital

University of Tampere

Amos Pasternack University Central Hospital

Iceland

University of Reykjavik

S Samuelsson University Hospital

Norway

University of Oslo

Knut Aas Ullevål Hospital
Christopher Juel Bjerkelund Aker Hospital
Jens Dedichen Rikshospitalet
Erik Enger Ullevål Hospital
Egil Gjone Rikshospitalet
Arvid John Hellem Akershus Central Hospital
Erik Kåss Revmatisme Hospital Oslo
Helge Laake Ullevål Hospital
Henrik Finne Lange Krohgstötten Hospital
Carsten Muller Ullevål Hospital
Ole Storstein Rikshospitalet
Harald Frey Aker Hospital
Trygve Wessel Aas Rikshospitalet

University of Bergen

Ole Jacob Broch Haukeland Hospital
Johs Boe Haukeland Hospital
Jarle Ofstad Haukeland Hospital

University of Tromso

Arne Nordóy Central Hospital

University of Trondheim

Rolf Rokseth Regionsykehuset i Trondheim
Jon Lamvik Regionsykehuset i Trondheim

Sweden

Karolinska Institutet Stockholm

Gunnar Biörck Serafimerlasarettet
Gunnar Birke Huddinge Hospital
Lars Erik Böttiger Karolinska Hospital
Rolf Luft Karolinska Hospital esp Endocrinology

University of Uppsala

Harry Bostrom University Hospital

University of Lund

Anders Gustafsson University Hospital
Bertil Hood Malmö General Hospital
Bernt Hokfelt Malmö General Hospital esp Endocrinology
Åke Nordén Dalby Lakarstation esp Community Medicine

University of Goteborg

Per Bjorntorp Sahlgren s Hospital
Hårje Bucht Sahlgren s Hospital esp Nephrology
Leif Hallberg Sahlgren s Hospital

University of Umeå

Per O Wester University Hospital

University of Linkoping

Sven Almqvist University Hospital

Acta Med Scand 202 163–171 1977

Free Fatty Acids in Plasma and Platelets Following Low and High-Dose Heparin during Alimentary Hyperlipaemia

Arne Nordøy Harald Vik Mo Ole D Mjøs and Roar Johnsen

From the Departments of Medicine and Physiology Institutes of Clinical Medicine and Medical Biology University of Tromsø Tromsø Norway

ABSTRACT A high caloric meal composed of mainly saturated or unsaturated fatty acids was given to 10 healthy male subjects Three hours later, during the period of alimentary hyperlipaemia with high plasma triglyceride levels they were given 5000 U heparin s c or 15000 U heparin i v During the subsequent three hours plasma and platelet triglycerides plasma and platelet FFA and platelet number were registered A marked increase in plasma FFA was observed on both occasions, most pronounced initially after the high dose heparin The concentrations were still markedly increased after both dosages three hours after administration The platelet FFA also showed a moderate but significant increase at the end of the experiment Both plasma and platelet FFA reflected the fatty acid composition of the dietary fatty acids After high-dose heparin, a positive correlation was established between the plasma TG level at the time of heparin administration and the subsequent increase in plasma FFA A transient drop in the number of circulating platelets was registered after high dose heparin In 6 patients with thromboembolic disorders treated with a continuous i v heparin infusion no marked changes were observed in plasma TG or plasma FFA after an ordinary breakfast The possible harmful effects of high levels of FFA are discussed It is suggested that the actual plasma TG level should be estimated by inspection of plasma before heparin treatment is initiated

High concentrations of plasma free fatty acids (FFA) during acute myocardial infarction (AMI) in man have been associated with a higher frequency of ventricular arrhythmias and sudden death (7 21) although others have failed to establish such a connection (25) Reduction of the plasma concentration of FFA by means of a nicotinic acid analog during the first 5 hours after myocardial infarction has been shown to reduce the occurrence of ventricular arrhythmias (22) Heparin given i v produces an elevation of plasma concentrations of FFA by liberation of the enzyme lipoprotein lipase from various organs (10) In experimental studies elevation of FFA by infusion of a triglyceride (TG) emulsion and heparin has been shown to increase the myocardial oxygen requirement (13) increase the size of an acute myocardial ischaemia (9 14) and induce ventricular arrhythmias in dogs during myocardial ischaemia (12) Although any deleterious myocardial effects of heparin administration during AMI in man have not yet been documented (16 24 26) the use of heparin in this situation is questionable because of the increase in plasma concentrations of FFA (11)

It is known that fatty acids both in vitro and in vivo may induce platelet aggregation and increase platelet adhesiveness (17) Experimental studies indicate that a concentration of plasma FFA of about 1200 μmol/l may be critical for platelet function (8) and the affinity of certain fatty acids like stearic and oleic acids to platelets is higher than for other fatty acids (18 28) Thus both the quantity and the quality of plasma FFA may have a bearing on the interaction with platelets When heparin is given i v during alimentary hyperlipaemia a marked increase in plasma FFA concentration occurs (19) Since heparin is widely used in clinical situations in various doses and administered subcutaneously or intravenously the present study was undertaken to examine the effect of heparin on

Table I *Effect of heparin 15 000 U i v or 5 000 U s c and saline 3 ml i v on plasma triglycerides (TG) and free fatty acids (FFA) (mmol/l) 3 hours after a saturated fatty meal (mean±S D of ten experiments)*

Time after injection (min)	Heparin (15 000 U i v)		Heparin (5 000 U s c)		Saline (3 ml i v)	
	TG	FFA	TG	FFA	TG	FFA
Before	2 67±1 2	0 91±0 32	2 72±0 99	0 83±0 48	2 84±0 81	0 86±0 21
15	2 67±1 31	4 72±1 48*	2 60±1 09	1 35±0 69*	2 70±0 88	1 00±0 [illegible]0
30	2 55±0 98	5 26±1 42*	2 64±1 06	1 81±0 62*	2 72±0 89	0 96±0 23
180	1 43±0 47	1 67±0 58**	1 88±0 60**	1 45±0 40	1 53±0 48*	0 85±0 17

Significance of difference compared with pretreatment values $^*p<0\ 01$ $^{**}p<0\ 05$

plasma and platelet FFA and platelet number during alimentary hyperlipaemia in healthy subjects Furthermore we have studied the effect of heparin when given as a continuous i v drip to patients with thromboembolic disorders on the concentration of plasma FFA

STUDY POPULATION AND METHODS

Ten healthy male subjects aged 20–25 years were fasted for 14 hours They were then given a meal of porridge made with cream with a total fat content of 175 g On another day six of the students were given a similar isocaloric meal made of soybean oil The fatty acid compositions of the dietary fats were as follows cream 12 0 (3%) 14 0 (14%) 16 0 (36%) 18 0 (15%) 18 1 (31%) 18 2 (1%) soybean oil 16 0 (10%) 18 0 (4%) 18 1 (17%) 18 2 (60%) 18 3 (9%)

Each student was given the fatty meal on three separate days Two days on a regular diet were allowed between each test Three hours after completion of the meal heparin (Nyco Nyegaard Oslo Norway) was given as a single s c dose of 5000 U or i v dose of 15 000 U On the third day a similar volume of saline was given i v The six subjects who got an unsaturated fatty meal were given heparin 5000 U s c 3 hours after completion of the meal

Six patients 3 males and 3 females aged 54–67 years were given a continuous heparin infusion as treatment for deep leg vein thrombosis Blood was collected fasting and 1 2 and 3 hours after a hospital breakfast comprising two pieces of bread a glass of milk and a cup of coffee The patients were given 30 000 U heparin in saline/24 hours as a continuous drip The last blood sample was collected fasting at least 24 hours after completion of heparin treatment

Preparation of test samples

Venous blood was collected in 5 ml samples in dried EDTA placed on ice and immediately centrifuged at 4 C for 10 min at 1000 *g* Plasma was pipetted off and stored at −20°C till testing within 10 days for plasma FFA triglycerides and albumin Blood samples of 4 5 ml were collected in 0 5 ml of 0 106 M citrate solution centrifuged for 10 min at 1 000 *g* and plasma was collected for estimation of activated partial thromboplastin time (Cephotest) Venous blood was collected in 36 ml samples in 4 ml of 0 077 M EDTA solution pH 6 4 and processed immediately Differential centrifugation yielded platelet-poor plasma (PPP) and platelet concentrate The platelet concentrate was washed three times in a washing fluid as described earlier (18) Aliquots of PPP and the platelet suspension which was frozen and thawed three times were used for lipid analysis

Platelet enumeration

Platelets were counted in whole blood collected in EDTA using isopycnic differential centrifugation (2) and a thrombocounter (Coulter Electr Ltd Dunstable Beds England)

Activated partial thromboplastin (APTT) time was measured using Cephotest (Nyco Nyegaard Oslo Norway)

Lipid analysis

Aliquots of plasma were estimated for fatty acids by the titrimetric method of Dole (3) as modified by Trout et al (29) and TG according to the method described by Eggstein and Kreutz (5) using a commercial kit (Boehringer Mannheim Mannheim Germany) All samples were tested in triplicate The coefficient of variation for the method of FFA measurement in plasma was 6% The possible influence of the sampling procedure on in vivo lipolysis was studied in separate experiments in samples drawn 30 sec after i v heparin injection in the hyperlipaemic state Samples cooled directly in N_2 and samples placed on ice for 15 sec showed no significant difference in plasma FFA concentrations Samples stored for 10 days at −20°C showed no significant changes in plasma FFA concentration Aliquots of PPP and platelet suspension were extracted with methanol and chloroform TG and fatty acids were separated by thin layer chromatography methylated and finally examined by gas–liquid chromatography which was performed on a F & M model 402 Hewlett Packard Gas Chromatograph as recently described (18)

Albumin in plasma was estimated according to the method of Doumas et al (14)

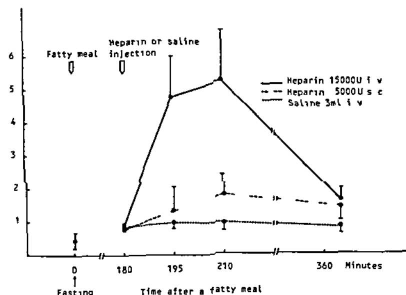


Fig 1 Concentration of plasma FFA in 10 healthy subjects given heparin i v or s c 3 hours after intake of a meal rich in saturated fatty acids

RESULTS

No untoward effects were observed in any of the test subjects The plasma albumin level was within normal limits in all subjects

The effect on plasma FFA and TG

Blood samples were collected in the fasting state three hours after a saturated fatty meal when an in tion of saline or heparin in various concentra tions was given and 15 30 and 180 min after the injection As demonstrated in Table I the fat ty meal had induced hypertriglyceridaemia three hours later followed by a decline towards normal values at the end of the experiment The reversal of the postalimentary hypertriglyceridaemia was not significantly different in subjects given heparin or saline i v A pronounced increase in FFA was observed after i v injection of 15000 U heparin A highly significant increase in plasma FFA was also observed after the low dose of s c heparin (Table I Fig 1) Three hours after a low or high dose of

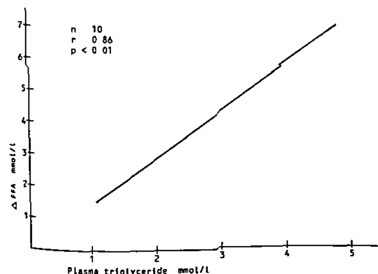


Fig 2 Correlation between plasma triglycerides at the time of i v injec tion of 15000 U heparin and the increase in plasma FFA 30 min after injection

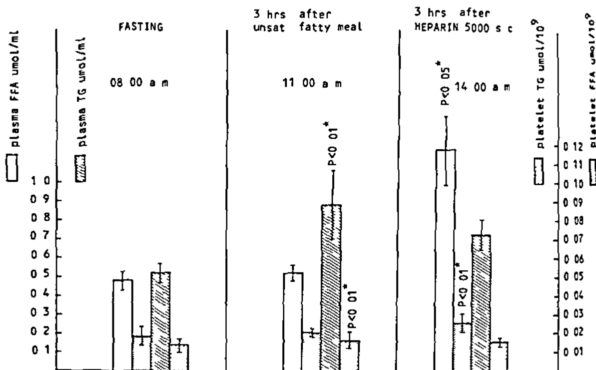


Fig 5 Plasma and platelet levels of TG and FFA in 6 healthy subjects fasting 3 hours after intake of a meal rich in unsaturated fatty acids and 3 hours after injection of 5000 U heparin s c given during alimentary hyperlipaemia S E M is given at the top of the columns

The effect of continuous heparin infusion plasma TG and FFA

6 patients on continuous heparin treatment for olic disorders blood samples were collected in the fasting state and hourly after an ordinary breakfast Another blood sample was collected in the fasting state more than 24 hours after discontinuation of the heparin treatment The TG level showed a very moderate but significant increase three hours after the meal (Table III) No significant changes were observed in the plasma FFA concentration during the combined effect of the meal and continuous heparin infusion (Table IV) All patients were within the therapeutic range as estimated by APTT time which was 2–4 times the normal When the last blood samples were collected the APTT times had normalized in all patients

Table IV *Plasma free fatty acid concentration (mmol/l) in 6 patients during heparin infusion before and after breakfast and without heparin after fasting for 24 hours or more*

Pat no	During heparin infusion				Without heparin (Fasting)
	Fasting	After a meal 1 h	2 h	3 h	
1	1 45	1 29	1 12	1 62	1 08
2	0 90	0 44	0 30	0 56	0 68
3	0 84	0 68	0 44	0 64	0 44
4	0 70	0 37	0 42	0 52	0 11
5	0 84	0 39	0 60	0 57	0 55
6	0 66	0 47	0 29	0 36	0 33
Mean	0 90	0 61	0 53	0 71	0 70
S D	0 29	0 35	0 31	0 45	0 33

DISCUSSION

In accordance with previous studies (19) a high dose of heparin given i v during alimentary hyperlipaemia effected a considerable increase in the plasma concentration of FFA Furthermore the present study demonstrates that even a low dose of heparin given s c during alimentary hyperlipaemia effected a marked increase in plasma FFA concentration Both with a low dose of heparin s c

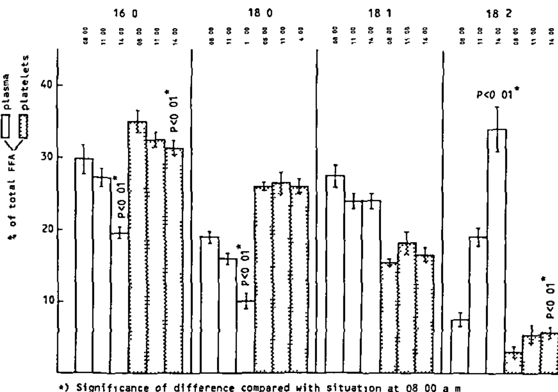


Fig 6 Distribution of the main fatty acids in plasma and platelet FFA in the fasting state (at 08 00) 3 hours after an unsaturated fatty meal (at 11 00) and 3 hours following 5000 U heparin s c (at 14 00) in 6 healthy subjects S E M is given at the top of the columns

and with a high dose i v the maximum increase in plasma FFA concentration was noted half an hour later However in both cases high concentrations of FFA were still present three hours after the injection of heparin

The significance of the observed effect of heparin on plasma concentrations of FFA is not known But in recent years there has been increasing evidence that in patients with AMI a high concentration of plasma FFA is associated with serious ventricular arrhythmias and sudden death (7 21) although other investigators have failed to establish such an association (25) The present study does not lend support to either point of view However concerning the use of low-dose heparin as an anticoagulant in patients with AMI it can be noted that even such a dose given s c was sufficient to increase the plasma concentration of FFA into the potentially harmful range for AMI patients It is of particular interest that subjects in the present study with the highest plasma TG concentration before heparin injection showed the most marked rise in plasma FFA concentration following heparin administration Thus one should perhaps reconsider the use of heparin treatment in subjects with a high plasma concentration of TG due either to a recent high fat intake or to a high concentration of very low density lipoproteins From a clinical point of view inspection of plasma before heparin is started would give valuable information

The present study confirmed that the increase in plasma FFA concentration mainly reflected an increase in those fatty acids which dominated in the fatty meal (19) Intake of a meal rich in saturated fatty acids with subsequent hypertriglyceridaemia injection of heparin and finally a high concentration of plasma FFA may all by a variety of mechanisms interfere with platelet function A meal rich in saturated fatty acids may by itself induce an increase in platelet factor 4 activity in plasma indicating that platelets have undergone a release reaction (20) Heparin per se has been shown to induce platelet aggregation (6) and in a recent prospective study on 52 patients receiving continuous

i v infusion of heparin 16 developed thrombocytopenia (1) Finally it has been established that saturated fatty acids in a concentration above the plasma albumin binding capacity may increase platelet adhesiveness and precipitate platelet aggregation and thrombus formation (17 27) In the present study except for a transient drop in platelet number 15 min after a high dose of i v heparin generally no significant changes were observed in the circulating number of platelets during the observation period This may indicate that an interaction between the various stimuli (TG heparin FFA) was present which counteracted their isolated effect Earlier studies have in fact shown that a small dose of heparin twice daily actually may prolong platelet survival (15) In accordance with previous findings (19 20) a significant rise in platelet TG was observed during alimentary hyperlipaemia Furthermore three hours after heparin injection high concentrations of plasma FFA were reflected in a small but significant increase in platelet FFA However since this effect was present after both a saturated and an unsaturated fatty meal (which does not interfere with platelet functions) the significance of the small increase in platelet FFA is not obvious The fatty acid pattern in platelet FFA reflected the plasma and dietary fatty acids

In the present patients with thromboembolic disorders treated with heparin as a continuous infusion only minor changes in plasma TG and free fatty acids were observed during the period following breakfast All patients were according to the estimated APTT times on adequate heparin treatment This lack of response compared with our test subjects must be attributed in the first place to the different amount and composition of the meals The test subjects were given a high caloric meal composed mainly of fats whereas the ordinary breakfast given to the patients is a low caloric meal with a low fat content In addition it seems that the age factor may influence the results in that the lipolytic activity induced by heparin is reduced in older subjects (23) Finally the effect of continuous administration of heparin on lipolytic activity may be different from that observed after bolus injection

REFERENCES

1 Bell W R Tomasuto P A Awring B M & Duffy T P Thrombocytopenia occurring during the administration of heparin Ann Intern Med 85 155 1976

2 de Brugere M Bellmot C & Sokal G Electronic platelet counting after isopycnic differential centrifugation A simple rapid and accurate method Thrombos Diathes Haemorrh 32 232 1974

3 Dole V P A relation between non esterified fatty acids in plasma and the metabolism of glucose J Clin Invest 35 150 1956

4 Doumas B T Watson W A & Biggs H G Albumin standards and the measurement of serum albumin with bromcresol green Clin Chim Acta 31 87 1971

5 Eggstein M & Kreutz F H Eine neue Bestimmung der Neutralfette in Blutserum und Gewebe I Prinzip Durchfuhrung und Besprechung der Methode Klin Wochenschr 44 262 1966

6 Eika G On the mechanisms of platelet aggregation induced by heparin protamine and polybrene Scand J Haematol 9 248 1972

7 Gupta D K Young R Jewitt D E Herzog M & Opie L Increased plasma free fatty acid concentrations and their significance in patients with acute myocardial infarction Lancet 2 1209 1969

8 Hoak J C Connor W E & Warner E D Thrombogenic effects of albumin bound fatty acids Arch Pathol 81 136 1966

9 Kjekshus J K & Mjøs O D Effect of inhibition of lipolysis on infarct size after experimental coronary artery occlusion J Clin Invest 5? 17?0 1973

10 Krauss R M Windmueller H G Levy R I & Fredrickson D S Selective measurement of two different triglyceride lipases in rat postheparin plasma J Lipid Res 14 286 1973

11 Kurien V A Yates P A & Oliver M F Free fatty acids heparin and arrhythmias during experimental myocardial infarction Lancet 2 185 1969

12 — The role of free fatty acids in the production of ventricular arrhythmias after acute coronary artery occlusion Eur J Clin Invest 1 225 1971

13 Mjøs O D Effect of free fatty acids on myocardial function and oxygen consumption in intact dogs J Clin Invest 50 1386 1971

14 Mjøs O D Kjekshus J K & Lekven J Importance of free fatty acids as a determinant of myocardial oxygen consumption and myocardial ischemic injury during norepinephrine infusion in dogs J Clin Invest 53 1290 1974

15 Mustard J F & Murphy E A Blood platelet economy during moderate and intensive heparin theraphy Blood 22 1 1963

16 Nelson P G Effect of heparin on serum free fatty acids plasma catecholamines and the incidence of arrhythmias following acute myocardial infarction Br Med J 3 735 1970

17 Nordøy A Platelets and fatty acids Biochem Exp Biol 11 295 1974/75

18 Nordøy A Bjørge J M & Strøm E Comparison of the main lipids in platelets and plasma in man Acta Med Scand 193 59 1973

19 Nordøy A Strøm E & Berntsen H The

of heparin on plasma and platelet lipids during alimentary hyperlipaemia in man Scand J Haematol 13 7? 1974

?0 Nordøy A Strøm E & Gjesdal K The effect of alimentary hyperlipaemia and primary hypertriglyceridemia on platelets in man Scand J Haematol 1? 329 1974

?1 Oliver M F Kurien V A & Greenwood T W Relation between serum free fatty acids and arrhythmias and death after acute myocardial infarction Lancet 1 710 1968

?? Rowe M J Neilson J M M & Oliver M F Control of ventricular arrhythmias during myocardial infarction by antilipolytic treatment using a nicotinic acid analog Lancet 1 295 1975

23 Rubegni M Bandinelli G & Proovedi D Heparin induced lipolysis in the elderly Lancet 2 903 1974

24 Russo J V Friesinger G C Margolis S & Ross R S Heparin and ventricular arrhythmias after myocardial infarction Lancet 2 1271 1970

25 Rutenberg H L Pamintuan J C & Soloff L A Serum free fatty acids and their relation to complications after acute myocardial infarction Lancet 2 559 1970

26 Saliba M J Jr Kuzman W J March D G & Lassy J E Effect of heparin in anticoagulant doses on the electrocardiogram and cardiac enzymes in patients with acute myocardial infarction A clinical pilot study Am J Cardiol 37 605 1976

27 Spector A A & Hoak J C Fatty acids platelets and microcirculatory obstruction Science 190 490 1975

28 Spector A A Hoak J C Warner E D & Fry G L Utilization of long-chain free fatty acids by human platelets J Clin Invest 49 1489 1970

29 Trout D L Estes E H Jr & Friedberg S J Titration of free fatty acids of plasma A study of current methods and a new modification J Lipid Res 1 199 1960

Acta Med Scand 202 173–177 1977

Nocturnal Hypertriglyceridemia and Hyperinsulinemia Following Moderate Evening Intake of Alcohol

Marja Riitta Taskinen and Esko A Nikkila

From Third Department of Medicine University Hospital Helsinki Finland

ABSTRACT The study was made to determine whether a moderate intake of alcohol in the evening influences the serum triglyceride and plasma insulin levels during the night and in the following morning Ethanol (1 5 g/kg) was given orally between 5 and 9 p m to 34 subjects The serum triglyceride and plasma insulin levels were followed during the night and the values compared with those taken after a sober evening The average maximal blood alcohol concentration was 1 1‰ (110 mg/dl) In 24 normolipidemic subjects, alcohol caused a definite nocturnal hypertriglyceridemia and hyperinsulinemia the mean maximal rise of serum triglyceride being 1 00 mM above the corresponding value for the control night In most cases the elevated triglyceride levels persisted until 8 a m next morning and 25% of the subjects would have been diagnosed as having a type IV hyperlipoproteinemia on the basis of these morning triglyceride values, taken after 11 hours' overnight fast In 10 patients with primary endogenous hypertriglyceridemia the mean maximal increase of serum triglyceride after ethanol was 3 65 mM and the average morning triglyceride value was still 2 40 mM higher than the corresponding level after a sober evening There was a highly significant correlation between the serum triglyceride and plasma insulin responses to ethanol ($r=+0$ 58 $p<0$ 001) It is concluded that conventional moderate drinking may be a common cause of hypertriglyceridemia whether occasional or more constant

It is well established that intake of ethanol may lead to elevation of the serum triglyceride concentration and that acute or prolonged consumption of substantial amounts of alcohol is one of the commonest causes of secondary hyperlipidemia in man (4 16) Alcoholic hyperlipemia is enhanced and prolonged by prior intake of fat (3 10 23) but otherwise little is known about the factors which determine the individual serum triglyceride response to alcohol In most studies alcohol has been administered to experimental subjects after an overnight fast (2 11 13 18 22) or as repeated small doses over a day (6 17) and there is little if any information on the mode of response to the most usual pattern of social alcohol consumption i e the intake of moderate amounts during evening hours after or with dinner

The present study was designed to examine the nocturnal and morning serum triglyceride and plasma insulin responses to moderate drinking of alcohol in the evening in normolipidemic and hyperlipidemic subjects

STUDY POPULATION

Twenty four healthy normolipidemic volunteers (age 20–33 years) and ten hyperlipidemic patients (age 22–42 years) participated in the study All normal volunteers had a fasting serum triglyceride level of less than 1 6 mM Three of these subjects were obese (26–37% over ideal body weight) The serum triglyceride level of the hyperlipidemic patients varied from 1 9 to 11 8 mM (mean 4 9±1 5) six had a type IV and four a type II b hyperlipoproteinemia Three of the patients were obese

Experimental schedule

The study was carried out in a metabolic ward where the subjects were given an isocaloric diet (alcohol excluded) during both experimental days A dinner was served at 4 30 p m and an evening snack at 8 00 p m The dinner contained approximately 700 calories of which 45% were from carbohydrates and 35% from fat The snack included one buttered sandwich and one cup of tea with sugar On the experimental day the subjects received 1 5 g of ethanol/kg b wt administered as three drinks of equal size containing 20% (w/v) ethanol in ice water and taken at 5 00 7 00 and 9 00 p m With this dosage blood ethanol concentration rose to a maximum at 11 00 p m and then

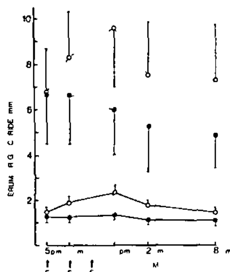


Fig 1 Mean serum triglyceride values during the night after either a sober evening (●) or intake of ethanol 1 5 g/kg (O) at times shown by arrows — normolipidemic subjects =hypertriglyceridemic patients The bars indicate S E M * $p<0 05$ ** $p<0 01$ ***=$p<0 001$ for the difference ethanol/control

ranged from 0 7 to 1 6 mg/ml (Λ) with a mean of 1 1 mg/ml At 8 00 a m next morning no alcohol was detectable in the blood

On the control day at least one day apart from the experimental day the subjects received similar meals but plain ice water instead of the alcoholic drinks Smoking physical activity and sleep were identical on the two days

METHODS

During both days blood samples were drawn at 5 00 7 00 11 00 p m 2 00 and 8 00 a m through an indwelling catheter placed in an antecubital vein Serum triglyceride (14) and plasma insulin (8) were assayed from each sample In six normal subjects the serum lipoproteins were isolated by ultracentrifugation (7) and analyzed for triglycerides and cholesterol (9)

RESULTS

The meals taken during a sober control day caused only a slight alimentary lipemia the mean maximal increase in serum triglyceride from the fasting (morning) value being 0 23 mM in normolipidemic and 1 80 mM in hyperlipidemic subjects Drinking of alcohol after dinner significantly increased the mean serum triglyceride levels of both groups of subjects for the whole night (Fig 1) The maximum of the postalcoholic nocturnal hypertriglyceridemia occurred at 11 00 p m in all subjects but at 8 00 a m next morning the mean serum triglyceride level was still significantly higher than the corresponding value after the control day The mean maximal increase in serum triglyceride caused by alcohol was 1 00 mM in normolipidemic and 3 65 mM in hyperlipidemic subjects The mean difference between the morning (fasting) triglyceride values after ethanol and control days was +0 31 mM and +2 40 mM in normolipidemic and hyperlipidemic subjects respectively (Fig 1)

The individual triglyceride responses of the normal subjects to alcohol are shown in Fig 2 After a sober control evening most individuals had a nocturnal serum triglyceride level of less than 2 00 mM whereas after an alcoholic evening 15 of the 4 volunteers developed a definite hyperlipemia during the night In six normal subjects (25 %) the morning triglyceride value after the ethanol day was above 1 60 mM and consequently they could thus have been diagnosed as having hyperlipidemia The individual triglyceride responses to ethanol showed a significant correlation to the basal triglyceride concentration (r +0 45 $p<0 01$)

Lipoprotein fractionation by ultracentrifuge indicated that the ethanol induced increase in triglyceride occurred in the VLDL fraction but in the morning sample the LDL triglyceride was also slightly elevated above the value for the control day (Table I) The cholesterol concentration in whole serum or in any of the lipoproteins was not changed after alcohol

The alcohol intake resulted in a slight but

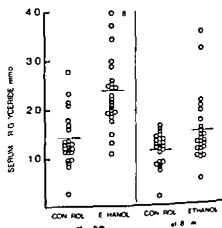


Fig 2 Individual serum triglyceride values of normolipidemic volunteers at *11* p m and *8* a m following a sober evening (control) or intake of ethanol during 5-9 p m

Table I *Ser im l poprotein cholesterol and tri gl ceride concentrat ons (mmol/l) follo ing drink ing of alcohol 1 5 g/kg at 5 9 p m*

	7 p m	11 p m	8 a m
Total triglyceride	1 1+0 3	? 1+0 4*	1 7+0 3**
VLDL	0 5+0 ?	1 6+0 3	0 9+0 3*
LDL	0 4+0 03	0 4+0 04	0 6+0 07*
HDL	0 ?+0 03	0 1+0 03	0 ?+0 03
Total cholesterol	4 8+0 03	5 5+0 04	5 5+0 03
VLDL	0 4+0 01	0 8+0 0?	0 5+0 0?
LDL	3 0+0 0?	3 2+0 03	3 5+0 03
HDL	1 4+0 0?	1 5+0 0?	1 5+0 0?

*$p<0.05$ * $p<0.01$ for the d fference from a value re co ded during a sober n ght

s gn f cant decrease in blood glucose levels during late n ght and early morning hours (3 0+0 03 vs 3 6+0 02 $p<0.01$) On the other hand the plasma nsul n level was s gnificantly h gher during the n ght after alcohol than the sober n ght (Fig 3) However at 8 00 a m next morn ng the alcohol effect on plasma insul n was no longer apparent The mean maximal postalcoholic increase in plasma nsul n in normolip demic and hyperlipidem c subjects was 1 9 fold and 3 8 fold respectively com pared with the values on the control day The magnitude of the postalcoholic rise of insulin was correlated to the basal plasma insul n level (Table II) and the response of triglyceride to ethanol (max mal or total) was related to the extent of the simultaneous rise of plasma insul n (Fig 4 and Table II)

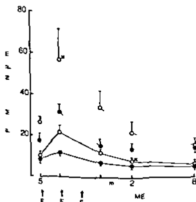
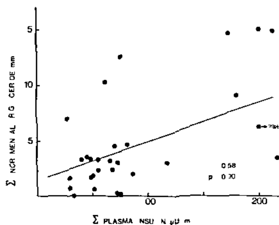


Fig 3 Mean plasma insul n values during the n ght after either a sober even ng or ntake of ethanol 1 5 g/kg at times marked by arrows Symbols as n F g 1



F g 4 Correlat on between postalcohol c response of plasma nsul n and max mal ncrement of serum triglyce de for all subjects stud ed

DISCUSSION

In this study alcohol was g ven to the experimental subjects after a normal d nner in amounts which were est mated to be common in people ac customed to social drinking habits i e to occa sional even ng drinks without getting drunk The degree of alcohol intoxication was sl ght or moder ate whether assessed by maximal blood alcohol

Table II *Relat ons bet een serum insulin and triglyceride parameters measured in 24 normo lipidem c and 10 hyperl pidem c subjects*

Maximal ncrements of IRI and TG are cal ulated by subtract ng correspond ng control values from post alcohol c values Sum of IRI values s calculated from absolute postalcohol c serum insul n values

x	y	r	p
Fast ng TG	Maximal ncrement of TG (ΔTG_m)	+0 45	<0 01
Fast ng IRI	Max mal increment of IRI (ΔIRI_m)	+0 65	<0 001
Fast ng IRI	Fast ng TG	+0 65	<0 01
Fasting IRI	Max mal increment of TG (ΔTG_m)	+0 48	<0 01
Fast ng IRI	Increment of fast ng TG (ΔTG)	+0 57	<0 001
Sum of IRI values (ΣIRI)	Maximal ncrement of TG (ΔTG_m)	+0 58	<0 001
Sum of IRI values (ΣIRI)	Sum of TG increments ($\Sigma\Delta$TG)	+0 58	<0 001
Sum of IRI ncrements ($\Sigma\Delta$IRI)	Sum of TG increments ($\Sigma\Delta$TG)	+0 40	<0 05

levels (average 1 1 ‰) or by clinical signs It is remarkable that with this dosage of ethanol a definite postalcoholic hypertriglyceridemia developed in the majority of subjects with normal basal serum lipid levels and that the hyperlipemia was markedly accentuated in patients with endogenous hypertriglyceridemia In one fourth of the normolipidemic subjects the fasting serum triglyceride level on the morning after alcohol intake was still elevated above the upper limit of the normal range and these persons could have been misclassified as having a type IV endogenous hyperlipidemia In this respect our results agree with the data of Ostrander et al (19) who in a population study found a definite relationship between the frequency of alcohol ingestion (by questionnaire) and the basal serum triglyceride concentration The present findings also support the conclusion drawn by Ostrander et al that moderate drinking is a more frequent cause of high serum lipid levels than generally assumed It is also probable that much of the spontaneous variations in serum basal triglyceride level and many transient hypertriglyceridemias can be attributed to use of alcohol rather than to variations in the composition of the diet Similarly the Monday morning hyperlipemia seen in clinical practice or in lipid clinics is probably accounted by weekend drinking rather than by excessive eating

The magnitude of the hyperlipemic response to alcohol is dependent on several factors including duration and degree of alcoholemia basal serum triglyceride level feeding state and previous diet In addition as yet unidentified individual factors may modify the response In several previous studies even relatively large daily amounts of alcohol have been found to be without effect on (fasting) serum triglyceride levels However in these cases ethanol has been administered in small repeated doses over several hours or throughout the whole day (6 12 17 20) and accordingly the blood alcohol level has remained relatively low When the dose of alcohol is increased (2) or the time of drinking shortened (11 12 21 22 23) an increase in the serum triglyceride level occurs regularly even in fasting subjects but in most of these instances the blood alcohol level has been raised above 1 5 mg/ml i e to values indicative of moderate or severe intoxication

Patients with endogenous hypertriglyceridemia show an exaggerated hyperlipemic response to ethanol although the average percentile increase of serum triglyceride level seems to be similar in normolipidemic and hyperlipidemic subjects In primary hypertriglyceridemia alcohol may increase the fasting serum triglyceride level at doses which do not influence the serum lipid levels of normal people (1 6 15 17) The great individual variability is indicated by the fact that in some cases an amount of alcohol constituting up to 40% of total daily calories has been reported to be without influence on serum basal triglyceride values (5 15) The lipemic response to ethanol seems to be independent of the amount of fat and carbohydrate in the basal diet (5 15) but when ingested together fat and alcohol potentiate each other s effect on the intensity of hyperlipemia

Drinking in the evening caused a rise of plasma insulin levels during the following night In spite of the correlation found between the postalcoholic increments of plasma insulin and triglyceride it is less probable that the acute ethanol induced hyperlipemia is caused by the preceding hyperinsulinemia On the other hand the elevated plasma insulin levels might contribute to the maintenance of high serum triglyceride levels during continued drinking Since both hyperinsulinemia and hypertriglyceridemia may promote atherogenesis alcohol can contribute to the development of cardiovascular disease

ACKNOWLEDGEMENTS

This study was supported by a grant from the Foundation for Alcohol Research in Finland (State Alcohol Monopoly Alko) and the Finnish State Medical Research Council (Academy of Finland)

REFERENCES

1 Amatuzio D S & Hay L J Dietary control of essential hyperlipemia Arch Intern Med 10? 1?? 1958
2 Avogaro P & Cazzolato G Changes in the composition and physico-chemical characteristics of serum lipoproteins during ethanol-induced lipaemia in alcoholic subjects Metabolism 24 1231 1975
3 Barboriak J J & Meade R C Enhancement of alimentary lipemia by preprandial alcohol Am J Med Sci 255 245 1968
4 Chait A Mancini M February A W & Lewis B Clinical and metabolic study of alcoholic hyperlipidaemia Lancet 2 62 1972
5 Erkelens D W The influence of alcohol and carbohydrates on hypertriglyceridaemia Thesis Groningen 1975

6 Ginsberg H Olefsky J Farquhar J W & Reaven G M Moderate ethanol ingestion and plasma triglyceride levels A study in normal and hypertriglyceridaemic persons Ann Intern Med 80 143 1974
7 Havel R J Eder H A & Bragdon J H The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum J Clin Invest 34 1345 1955
8 Herbert V Lau K S Gottlieb C W & Bleicher S J Coated charcoal immunoassay of insulin J Clin Endocrinol Metab 25 1375 1965
9 Huang T C Chen C P Wefler V & Raftery A A stable reagent for the Liebermann Burchard reaction Application to rapid serum cholesterol determination Anal Chem 33 1405 1961
10 Jaillard J Sezille G Fruchart J C Dewailly P Scherpereel P & Rault C Modification sous l effet de l alcool de l épuration lipidique plasmatique apres hyperlipemie induite par le repas gras ou les perfusions d emulsions lipidiques chez l homme Nutr Metab 15 232 1973
11 Jones D P Losowsky M S Davidson C S & Lieber C S Effect of ethanol on plasma lipids in man J Lab Clin Med 62 675 1963
12 Jones D P Perman E S & Lieber C S Free fatty acid turnover and triglyceride metabolism after ethanol ingestion in man J Lab Clin Med 66 804 1965
13 Kaffarnik H & Schneider J Zur kurzfristigen Wirkung des Athylalkohols auf die Serumlipide gesunder fastender Versuchspersonen Z Gesamte Exp Med 152 187 1970
14 Kessler G & Lederer H In Automation in analytical chemistry (ed L T Skeggs) p 341 Technicon Symposium 1966
15 Kudzma D J & Schonfeld G Alcoholic hyperlipidemia induction by alcohol but not by carbohydrate J Lab Clin Med 77 384 1971
16 Lieber C S Hepatic and metabolic effects of alcohol (1966 to 1973) Gastroenterology 65 821 1973
17 Mendelson J H & Mello N K Alcohol induced hyperlipidemia and beta lipoproteins Science 180 1372 1973
18 Nestel P J & Hirsch E Z Mechanism of alcohol induced hypertriglyceridemia J Lab Clin Med 66 357 1965
19 Ostrander L D Jr Lamphiear D E Block W D Johnson B C Ravenscroft C & Epstein F H Relationship of serum lipid concentrations to alcohol consumption Arch Intern Med 134 451 1974
20 Schlierf G Gunning B Uzawa H & Kinsell L W The effects of calorically equivalent amounts of ethanol and dry wine on plasma lipids ketones and blood sugar in diabetic and nondiabetic subjects Am J Clin Nutr 15 85 1964
21 Talbott G D & Keating B M Effects of preprandial whiskey on postalimentary lipemia Geriatrics 17 802 1962
22 Verdy M & Gattereau A Ethanol lipase activity and serum lipid level Am J Clin Nutr 20 997 1967
23 Wilson D E Schreibman P H Brewster A C & Arky R A The enhancement of alimentary lipemia by ethanol in man J Lab Clin Med 75 264 1970

Acta Med Scand 202 179–181 1977

Effect of Clofibrate and Calcium in Type II A Hyperlipoproteinaemia

A Lehtonen and J Viikari

From Department of Medicine University of Turku Turku Finland

ABSTRACT In type II A hyperlipoproteinaemia, treatment with cholestyramine (12 g) calcium clofibrate (2 g) and a combination of calcium clofibrate and calcium carbonate (2+2 g) reduced the cholesterol concentration The combination of calcium and clofibrate was at least as effective as cholestyramine Calcium clofibrate decreased very low density and low density lipoprotein cholesterol triglycerides and phospholipids whereas no changes were found in high density lipoproteins Cholestyramine decreased low density lipoprotein lipids

Hyperlipoproteinaemia is a common finding in patients with ischaemic heart disease (15) Numerous pharmacologic approaches to the treatment of primary type II hyperlipoproteinaemia have been tried (10 11) The most marked effect seems to be obtained with agents which interrupt the enterohepatic circulation of bile acids (8) such as cholestyramine Cholestyramine however is unacceptable to many patients (10) The cholesterol reduction with clofibrate in type II hyperlipoproteinaemia has been found to be smaller than that with cholestyramine (13) *Increased calcium ingestion* seems to cause a small decrease in cholesterol but the results concerning triglyceride levels are somewhat controversial (6 18)

The purpose of this study was to compare the therapeutic effects of cholestyramine calcium clofibrate and the combination of calcium carbonate and calcium clofibrate

SUBJECTS AND METHODS

The effect of cholestyramine (Questran* 12 g/day) was compared with calcium clofibrate (Bristol Myers Co 2 g/day) and with calcium clofibrate+calcium carbonate (2+2 g/day) in 12 clinically healthy patients (6 males 6 females aged 22–47 years) The patients were kept on a proper diet for at least 3 months before medical treatment and throughout the period of medication

All the patients had primary type II A hyperlipoproteinaemia with plasma cholesterol levels of at least 8 mmol/l with normal triglyceride levels (below 2 mmol/l) and with type II patterns of cellulose acetate electrophoresis of plasma lipoproteins at the time of selection

The study protocol is shown in Table I

Blood samples were drawn after an overnight fast for determination of cholesterol triglycerides and calcium

Lipoprotein isolation and fractionation were carried out by a density gradient centrifugation method which is described in detail elsewhere (17)

Lipoprotein fractions were dialysed against water for at least 4 h and lyophilized Lipids were extracted according to Folch et al (7) Cholesterol was determined with a modification of the ferric chloride method of Badzio and Boczon (1) Lipid phosphorus was assayed according to Bartlett (2) except that the digestion was made according to Svanborg and Svennerholm (16) Neutral glycerides were determined by the method of Carlson (5)

The following routine laboratory tests were performed before treatment and after each period of medical treatment Hb hematocrit RBC WBC urinalysis serum creatinine bilirubin alkaline phosphatase aspartate aminotransferase fasting blood sugar and phosphorus

RESULTS

All patients completed the periods of the study The changes in the concentrations of cholesterol and triglycerides during the medical treatment are shown in Fig 1

Calcium clofibrate clearly reduced the plasma concentrations of cholesterol and triglycerides There were no further decreases in these concentrations after the addition of $CaCO_3$ The mean serum cholesterol concentration also decreased during cholestyramine medication but there was instead a slight rise in the level of serum triglyceride concentration

The changes in the different lipoprotein fractions

Table 1 *Number of women sampled for and participating in the different investigations and their body weights (mean±S D)*

Age group		24 hour recall		Dietary history		24 hour urine collected	
No	y	Participating/ sampled (*n*)	B wt (kg)	Participating/ sampled (*n*)	B wt (kg)	Participating/ sampled (*n*)	B wt (kg)
1	38	336/372	63 1±10 5	74/83	62 7±11 7	73/88	63 6±10 9
2	46	400/431	62 9± 9 8	96/101	63 2±10 8	90/97	63 7± 9 1
3	50	372/398	66 3±11 3	117/119	68 1±12 6	356/398	68 1±12 5
4	54	176/180	65 9±12 6	95/97	66 1±12 2	165/176	64 7±11 8
5	60	77/81	66 3±11 1	36/37	67 7±11 9	71/81	68 3±10 1
Total		1 361/1 462		418/437		755/835	

Significance of differences between groups (Student s t test)

24 hour recall	Dietary history
1–3 $p<0\ 01$	1–3 $p<0\ 01$
1–4 $p<0\ 01$	
1–5 $p<0\ 05$	1–5 $p<0\ 05$
2–3 $p<0\ 01$	2–3 $p<0\ 01$
2–4 $p<0\ 01$	2–4 $p<0\ 01$
2–5 $p<0\ 01$	2–5 $p<0\ 05$

terviews should cover all days of the week so as to be representative for the mean consumption of the group investigated Instead a weighed mean value for each age group covering seven days a week was calculated as follows twice the mean intake on Sundays was added to five fourths of the sum of the mean intake per weekday The total was then divided by seven A questionnaire adapted for this study was used in the dietary history interviews and has been described briefly elsewhere (3)

The intakes of energy and nutrients were calculated from the data collected using a computer system modified by one of the authors (B K L) after Westin (13) The system was based mainly on the food composition tables of Abramson (1) The sucrose content of the diets was calculated separately and does not include naturally occurring sucrose in fruits and vegetables All the dietary data were quantified and coded using a fixed size for small ordinary and large servings respectively To

ı Mean energy and nutrient intake in five age groups of women as calculated from 24 hour recall ns

Age group No	y	*n*	Energy (kcal)	Protein (g)	Fat (g)	Total CHO (g)	Sucrose (g)	Ca (g)	Fe (mg)	Vitamins (mg) A	B₁	B₂	C
1	38	336	1 630	58	72	171	37	0 8	12	0 94	1 0	1 4	69
S E			27	1 1	1 6	3 2	1 4	0 02	0 3	0 05	0 02	0 04	3
2	46	400	1 600	57	71	167	34	0 8	12	0 96	1 1	1 4	75
S E			30	1 0	1 5	4 1	1 3	0 02	0 3	0 05	0 03	0 05	4
3	50	372	1 520	54	67	161	36	0 7	11	0 87	1 0	1 3	70
S E			28	1 0	1 5	3 2	1 6	0 02	0 2	0 05	0 02	0 04	3
4	54	176	1 515	56	67	158	34	0 7	11	0 77	1 1	1 2	65
S E			42	1 6	2 4	4 4	1 9	0 03	0 3	0 05	0 04	0 04	4
5	60	77	1 410	52	61	151	32	0 7	11	0 80	0 9	1.2	77
S E			48	2 0	2 8	5 6	2 4	0 04	0 6	0 08	0 05	0 09	7

Significance of differences between groups (Student's t test)

	Energy	Protein	Fat	Total CHO	Sucrose	Ca	Fe	A	B₁	B₂	C
1–2	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
1–3	$p<0\ 01$	$p<0\ 02$	$p<0\ 01$	$p<0\ 05$	ns	$p<0\ 05$	$p<0\ 03$	ns	ns	ns	ns
1–4	$p<0\ 025$	ns	$p<0\ 05$	$p<0\ 025$	ns	ns	$p<0\ 02$	$p<0\ 025$	ns	ns	ns
1–5	$p<0\ 001$	$p<0\ 02$	$p<0\ 005$	$p<0\ 005$	ns	$p<0\ 05$	ns	ns	ns	ns	ns
2–3	ns	$p<0\ 05$	$p<0\ 05$	ns	ns	ns	ns	ns	$p<0\ 05$	ns	ns
3–4	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
4–5	ns	ns	ns	ns	ns	ns	ns	ns	ns		

Table III *Mean intake of energy and some nutrients in five age groups of women as calculated from dietary history interviews*

Age group No	y	n	Energy (kcal)	Protein (g)	Fat (g)	Total CHO (g)	Sucrose (g)	Ca (g)	Fe (mg)	Vitamins (mg) A	B_1	B_2	C
1	38	74	2 065	73	91	226	48	1 0	15	1 53	1 35	1 84	112
S E			54	1 7	3 1	6 6	3 8	0 05	0 4	0 06	0 04	0 07	7
2	46	96	2 105	74	91	234	50	1 0	15	1 45	1 36	1 80	119
S E			57	1 6	3 1	6 6	3 1	0 04	0 3	0 05	0 03	0 05	7
3	50	117	2 040	74	86	230	45	1 0	16	1 40	1 41	1 80	108
S E			55	1 8	2 7	7 5	2 4	0 04	0 5	0 05	0 04	0 06	5
4	54	95	1 965	71	84	219	47	1 0	14	1 30	1 34	1 67	112
S E			49	1 6	2 6	5 9	3 1	0 04	0 3	0 05	0 02	0 06	7
5	60	36	1 870	68	79	211	42	0 9	14	1 17	1 25	1 52	106
S E			73	2 4	4 1	9 4	4 5	0 06	0 5	0 05	0 05	0 09	11
Significance of differences between groups (Student s t test)													
1-2			ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
1-3			ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
1-4			ns	ns	ns	ns	ns	ns	ns	$p<0$ 001	ns	ns	ns
1-5			$p<0$ 05	ns	$p<0$ 02	ns	ns	ns	ns	$p<0$ 001	ns	$p<0$ 01	ns
2-5			$p<0$ 025	$p<0$ 05	$p<0$ 05	ns	ns	ns	ns	$p<0$ 001	ns	$p<0$ 01	ns
3-5			ns	ns	ns	ns	ns	ns	ns	$p<0$ 01	$p<0$ 02	$p<0$ 01	ns
4-5			ns	ns	ns	ns	ns	ns	ns	$p<0$ 05	ns	$p<0$ 01	ns

evaluate the dietary intake the data were compared with a guide to the evaluation and planning of diets by Eeg Larsen et al (5)

Urine analysis In another randomized subsample (n= 835) an individual 24-hour urine collection was made Oral and written instructions were given to the partici pants at the first interview and they received a 5 l plastic flask for collection of the urine at home and at work An analysis for urinary nitrogen was performed using the Technicon AutoAnalyzer Method N 3b In normal indi viduals in nitrogen balance the mean daily nitrogen intake is close to the mean 24-hour urinary nitrogen plus 2 g an arbitrarily chosen figure for daily dermal and faecal nitro- gen losses (9) The nitrogen intake values were converted to the corresponding protein intake values by multiplica tion with the factor 6 25

RESULTS

The number of women in each age group sampled and participating in the different investigations together with their mean body weights are given in Table I No 24 hour recall data were obtained in 101 women of whom 91 refused to take part in the dietary interview In the remaining 10 women the interview was unsuccessful because of incoopera bility Nineteen dietary history interviews were missed owing to refusals (n=14) or communication difficulties (n=5) Eighty out of 835 women refused to participate in the 24 hour urine collection

24 hour recall

The mean comsumption of energy and nutrients in the five groups of women calculated from the 24 hour recall method is given in Table II Ap- proximately one third of the women interviewed stated that their consumption during the last 24 hours was non typical When comparing the typical (n=267) and the non typical (n=123) days in one of the age groups (46 years) no statisti cally significant differences were found for energy ($p<0$ 50) protein ($p<0$ 10) fat ($p<0$ 90) or carbo- hydrates ($p<0$ 20)

There were no significant differences in the mean energy intake between single days of the week The calculated weighed mean value gave an energy intake of only 0 25% above the observed energy value In the following therefore only the observed uncorrected mean values for energy and nutrients will be given

The consumption of *energy protein fat* and *carbohydrates* decreased gradually and significant ly with increasing age Compared with the age group 38 years the *calcium* supply was significant ly decreased only in women 50 and 60 years of age as was the *iron* content of the diet in women 50 and 54 years old Of the *vitamins* investigated a signifi

Table IV *Mean daily urinary nitrogen losses and intake of protein estimated as 6 25 (N_{urine}+2) (9) in five age groups of women*

Age group			Urinary N (g/day)		Estimated intake of protein (g/day)	
No	*y*	*n*	*Mean*	*S E*	*Mean*	*S E*
1	38	73	10 0	0 31	75	1 9
2	46	90	10 0	0 32	75	2 0
3	50	356	10 0	0 15	75	0 9
4	54	165	9 7	0 21	73	1 3
5	60	71	10 0	0 32	75	2 0

cant decrease in vitamin A intake was found only in women 54 years old When comparing the other age groups with each other significant differences were found only in a few instances (Table II)

Dietary history

The mean consumptions of energy and certain nutrients obtained with the dietary history method are given in Table III The daily *energy* supply was essentially the same in four age groups (2 065 2 105 2 040 1 965 kcal respectively) Women 60 years old consumed significantly less energy (1 870 kcal) than those in the two youngest age groups The consumption of *nutrients* did not differ between age groups except that 60-year old women consumed gnificantly less protein fat retinol and ribo-vin than the youngest women A mean daily *sucrose* content of approximately 40–50 g was found in the diets of women in all age groups No age related differences were found

24 hour urine collection

The mean protein intake calculated from urine analyses is given in Table IV There were no significant differences between the age groups

Comparison of data

The 24 hour recall method gave significantly lower values for the intake of energy and nutrients than the dietary history method As can be seen from Tables II and III the mean energy supply estimated by dietary history was approximately 30% above that of the 24 hour recall The mean percentage deviations for protein fat carbohydrates sucrose calcium and iron were 30 ($p<0$ 001) 28 ($p<0$ 001) 39 ($p<0$ 001) 33 ($p<0$ 05) 31 ($p<0$ 005) and 39 ($p<0$ 005) respectively

Protein calculated from urinary nitrogen deviated insignificantly from the dietary history figures by only 1 1–3 6% ($p<0$ 40) in the age groups 38–54 years and by 10% ($p<0$ 05) in women 60 years of age The deviation from the 24 hour recall data was highly significant ($p<0$ 001) in all age groups

Evaluation of nutrient intakes

When evaluating the diet of the women (Fig 1) only the data from the dietary history interviews were used Table V outlining the standards for this evaluation is derived from the three step scale guide by Eeg Larsen et al (5)

The *protein* supply was acceptable or high in most women studied and below the acceptable level in 27 out of 418 women (6 4%) In all age groups there was a high incidence (71–89%) of an excessive intake of *fat* expressed as % of the total energy supplied Fat contributed less than 25% of energy in only 5 out of 418 women (1%) The number of women having an acceptable intake of fat differed between the age groups (χ^2=10 785 d f =4, $p<0$ 05) Significantly fewer women in age groups 50 and 60 had too high a fat intake (χ^2= 8 289 d f =1 $p<0$ 01)

The *calcium* intake was acceptable or high in 78–89% of the women in the various age groups in total 72 (17%) had less than 0 6 g Ca per day An intake below the minimum level was found in 18 (4%) out of 418 women The *iron* intake was acceptable in only 6 (8%) of the youngest women The corresponding percentages in the other two groups of fertile women were 16 and 20 respective-

Table V *Standards for evaluating the diet of women aged 38–60 based on the figures given by Eeg Larsen et al (5)*

	Nutrient intake		
	Recom mended	Accept able	Mini-mum
Protein (g/kg)	≥0 9	≥0 8	≥0 7
Fat (cal %)	25–35	≤35	25
Calcium (g/d)	≥0 8	≥0 6	≥0 4
Iron (mg/d)			
Age group 38–50			≥15
Age group 54–60			≥10
Retinol (mg/d)	≥0 75	≥0 6	≥0 4
Thiamin (mg/1 000 kcal)	≥0 6	≥0 4	≥0 3
Riboflavin (mg/1 000 kcal)	≥0 9	≥0 7	≥0 5
Ascorbic acid (mg/d)[b]	≥60	≥40	≥30

[a] Hällstrom (8) studying the same population of women found that of those 54 years of age only 2% were still fertile
[b] In raw unprocessed foods

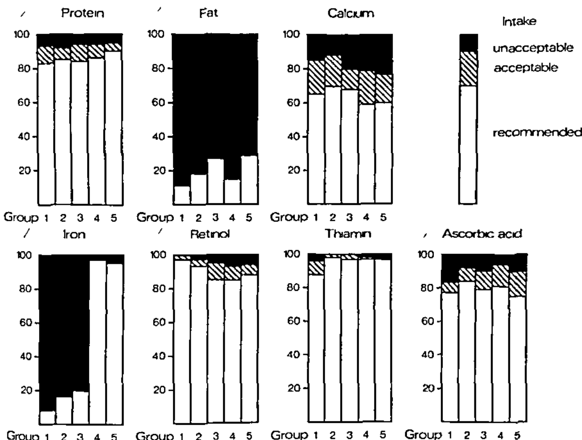


Fig 1 Percentage of women in five age groups having a recommended acceptable or unacceptable intake of protein fat calcium iron retinol thiamin and ascorbic acid

ly Of elderly women 95–98% had an acceptable iron supply

The dietary intake of *retinol* was acceptable or high with a few exceptions Only one woman 50 years old had a retinol supply below minimum 17 were below the acceptable level Likewise there was only one women of the 418 studied whose diet was below minimum for *thiamin* and only 6 were below the acceptable level In most women the *ascorbic acid* content of the diet was acceptable or high but in 6 (1 5%) out of 418 women the intake of this vitamin was below the minimum level and 35 (8%) took less than the acceptable quantity

DISCUSSION

The present paper presents the dietary characteristics in selected age groups of women Two different interview methods 24 hour recall and dietary history were used and the results are compared with estimates of protein based on urinary nitrogen excretion (9) Although both interview methods were found to be usable for a rough estimate of nutrient density i e amount of nutrient per energy unit the validity test of urinary nitrogen excretion showed that for quantitative purposes dietary history was the method of choice The significant discrepancy between urinary nitrogen and protein intake calculated from the dietary history of the oldest women may be attributed to the rather small number of women interviewed in this age group In a similar dietary study on 370 participants aged 70 there was very good agreement between the two calculations (12)

The data for energy intake obtained with the dietary history method in the different groups of women were in accordance with standards (5 6) and the values did not decrease until the age of 60 Thus most women belonged to the so-called low energy consumer group We question the data

obtained by means of the 24 hour recall method although they agree with previous population studies on women in Sweden (2 7) where the same interview method had been used

The age-dependent changes in body weight observed in this study are in accordance with previous findings on women in Gothenburg (11) and indicate a decreased physical activity with age The body compositions of the women in the different age groups have been determined with isotope dilution methods and will be described elsewhere

In 1969 the mean sucrose intake in Sweden based on statistics was about 120 g per person and day (Westin personal communication) Thus the sucrose consumption in our material amounted to less than half of this value According to Yudkin (14) sugar consumption differs within populations and is dependent on sex and age men consuming more sugar than women young people more than old Moreover part of the intake of sucrose through sweets and beverages may have been overlooked during the interviews

When comparing the nutrient density of the diets the results from both interview methods used here did agree with the data from the previous studies (2 7) except for iron This may possibly be attributed to an increased iron fortification of wheat r in the years between the present and the pre s studies

Judged from the dietary history results there were no age related changes in calcium or iron intake In the age group 50 years only 27% had regular menstruations (8) which indicates that for about two-thirds of these women the iron intake could be satisfactory

The present study supports the opinion (10) that the dietary intakes of protein calcium retinol thiamin and ascorbic acid are most often satisfactory in middle aged Swedish women but that their intake of iron is too low and of fat too high

All women subjected to the dietary history method were invited to participate in a new population study during 1974–75 This study will allow us to assess any possible changes in food habits and their nutritional implications in the interval during which a governmental campaign for improving food habits ("Diet and Exercise Campaign) has taken place in Sweden

ACKNOWLEDGEMENT

This part of the population study was supported by grants from the Swedish Margarine Manufacturers Association Fund for Nutrition Research and the Swedish Medical Research Council (project 19X 570)

REFERENCES

1 Abramson E Kosttabell 5th ed Laromedelsf rlagen Stockholm 1971
2 Ahlmark A Kim D Ryd G & Wretlind A Kostundersokningar av kollektivanstallda vid en verkstadsindustri Arbetsmedicinska institutet Stockholm AI rapport no 17 1971
3 Arvidsson Lenner R & Blohme G Dietary habits and body composition in a population sample of women aged 50 with special reference to decreased glucose tolerance and/or subnormal early insulin response Acta Med Scand (Suppl) 566 76 1974
4 Bengtsson C Blohme G Hallberg L Hallström T Isaksson B Korsan Bengtsen K Rybo G Tibblin E Tibblin G & Westerberg H The study of women in Gothenburg 1968–1969—A population study General design purpose and sampling results Acta Med Scand 193 311 1973
5 Eeg Larsen N Isaksson B Nicolaysen R & Wretlind A Veiledning til vurdering og planlegging av kosthold Landsforeningen for kosthold og helse Universitetsforlaget Oslo 1971
6 Food and Nutrition Board Recommended dietary allowances 8th revised edition 1974 National Academy of Sciences Washington D C 1974
7 Hallberg L Fetma hos kvinnor i olika åldrar Kaloriintag och födoamnesval Lakartidningen 63 61? 1966
8 Hallström T Mental disorder and sexuality in the climacteric A study in psychiatric epidemiology Thesis Orstadius Boktryckeri Göteborg 1973
9 Isaksson B Totalkvave i urin som mått på proteinintaget Medicinsk Riksstamma Stockholm 29 november 1970 Sammanfattningar s 3-4 nr 7 To be published in English
10 Johansson M & Wretlind A Kostundersökningar i Sverige Naringsforskning 17 52 1973
11 Lewin T & Jonsson B Some somatometric data on Swedish females aged 15–70 years Part II Acta Morphol Neerl Scand 11 187 1973
12 Steen B Isaksson B & Svanborg A Intake of energy and nutrients and meal habits in 70-year-old males and females in Gothenburg Sweden A population study Under publication 1977
13 Westin S I The use of electronic data processing technique on dietary research National Institute of Public Health Nutrition Division Stockholm 1964
14 Yudkin J Sucrose in the aetiology of coronary thrombosis and other diseases In Sugar (ed J Yudkin J Edelman & L Hough) p 231 Butterworths London 1971

Acta Med Scand 202 189–191 1977

Renal Venous Output of Kinins in Patients with Hypertension and Unilateral Renal Artery Stenosis

U L Hulthen H Lecerof and B Hökfelt

From the Departments of Endocrinology and Clinical Physiology University of Lund Malmo General Hospital Malmo Sweden

ABSTRACT In four patients with hypertension and angiographically pronounced unilateral renal artery stenosis, kallikrein activity was estimated in each kidney separately by the determination of kinin output in the renal veins All patients showed suppression of renin release from the kidney with a non stenotic artery Accordingly plasma flow from the kidney with artery stenosis could be estimated The ratio of venous output of kinins between the kidney with a non stenotic artery and the one with artery stenosis was 2 6–6 5 This indicates that renal artery stenosis leads to diminished intrarenal kinin generation Reduced kinin formation may explain the low diuresis and natriuresis found in the kidney with artery stenosis

Kallikreins generate kinins (18) known to increase renal blood flow diuresis and natriuresis (2) A positive correlation between urinary excretion of kallikrein most likely of renal origin (15) and sodium has been reported in man by Adetuyibi and Mills (1) Renal artery stenosis is known to reduce urine flow and sodium excretion (4) Margolius et al (10) found no significant difference in urinary kallikrein excretion in patients with renal artery stenosis as compared with normal subjects

In the present study we have estimated the kallikrein activity in each kidney separately in hypertensive patients with unilateral renal artery stenosis by determining the kinin output in the renal veins

PATIENTS AND METHODS

Four patients with hypertension in WHO classification stages I–II and with angiographically pronounced unilateral renal artery stenosis were studied Essential clinical data are presented in Table I Patient 3 had maturity onset diabetes mellitus of three years duration well controlled by dietary means All patients became normotensive after reconstructive vascular surgery

Before renal vein sampling the patients had been off antihypertensive medication for at least one week They were kept in bed and fasted overnight prior to catheterization Radiopaque polyethene catheters with one tip hole and two side holes 5 mm from the tip (Odman Ledin size 8F KIFA Sweden) were inserted by Seldinger technique from the femoral veins and advanced into the renal vein on each side under fluoroscopic control Correct position of the catheters was also ensured by analysis of the oxygen tension in the renal veins as compared with that of the inferior caval vein Another polyethene catheter PE 160 (Intramedic® Clay Adams New York) was inserted into an antecubital vein and advanced cephalic about 20 cm Samples for determination of plasma renin activity (PRA) and kinin concentration were drawn simultaneously from the renal veins followed immediately by a sample for assay of PRA from the brachial vein Kinin concentration was not assayed in blood from the brachial vein because we have found that an indwelling catheter in peripheral veins often activates blood kallikrein (6)

Blood kinins were determined using a radioimmunoassay in which kallidin showed 97% reactivity as compared with bradykinin and major degradation products of kinins less than 0 01% cross reactivity (7) Thus the figures obtained represent the sum of the concentrations of bradykinin and kallidin For each patient all samples taken were measured in one and the same single assay run The intra assay coefficient of variation for the means of triplicates was 12 6%

PRA was measured as the amount of angiotensin I generated following three hours incubation (5) Again all samples from each patient were measured in one and the same single assay run The intra assay coefficient of variation for the means of duplicates was 10%

When no renin is secreted by the kidney with a non stenotic artery (see below) plasma flow from the kidney with artery stenosis can be estimated by applying an equation used earlier by Sealey et al (16) In this equation we have used our finding that mean PRA was 30% higher in the renal veins compared with a peripheral vein in supine position in 13 patients with essential hypertension and

Table I *Essential clinical data on the patients*

	Pat 1	Pat 2	Pat 3	Pat 4
Age (y) and sex	20 ♀	32 ♀	62 ♀	42 ♂
Renal angiographic findings	Left artery stenosis min diam 1 0–1 5 mm post stenotic dilatation collateral circulation	Right artery fibromuscular dysplasia min diam 1 5 mm	Left artery stenosis min diam 1–2 mm poststenotic dilatation	Left artery stenosis min diam 1–2 mm poststenotic dilatation
Kidney size (cm)				
Right	14 5×6	12 5×6 5	15×7	16×6
Left	14×6	13 5×6 5	10×5	13×6
Serum creatinine (μM/l)	69	80	98	102
Creatinine clearance (ml/min 1 73 m²)	158	162	85	90
Supine BP (mmHg)				
Preoperative	160/110	180/110	200/110	180/120
Postoperative	125/80	130/85	160/85	140/90

normal renal function (8) Assuming a plasma flow of 300 ml/min from the kidney with a non stenotic artery the ratio of venous output of kinins between the kidneys could be calculated

RESULTS

All patients showed suppression of renin release from the kidney with a non stenotic artery PRA in renal vein being 98–109% of the values found in peripheral vein In the vein from the kidney with artery stenosis PRA was 228–320% compared with peripheral vein (Table II) These findings indicate a hemodynamic significance of the renal artery stenosis (17)

Plasma flow from the kidney with artery stenosis estimated as described above ranged from 82 to 141 ml/min The ratio of venous output of kinins between the kidney with a non stenotic artery and the one with artery stenosis was 2 6–6 5 (Table II) Thus the output of kinins was significantly lower from the kidney with artery stenosis

DISCUSSION

The finding of a reduced venous output of kinins from the kidney with artery stenosis is in accordance with results concerning urinary kallikrein excretion in dogs Bevan et al (3) reduced renal artery pressure by a constricting clamp and found a highly positive correlation between urinary kallikrein excretion and renal artery pressure Keiser et al (9) found that urinary kallikrein excretion from the kidney with a ligated artery was significantly lower than from the contralateral kidney

There is good evidence that renal venous output of kinins reflects renal kallikrein activity During infusion of bradykinin into a renal artery in dogs the average amount of bradykinin recovered in the renal vein was less than 15% (14) In intact dogs acute intragastric saline loading increased the concentration of kinins in the inferior caval vein cephalic to the renal veins as well as the urinary excretion of kallikrein whereas saline loading in nephrectomized animals if anything slightly decreased the caval concentration of kinins (12)

Several studies support the view that intrarenal kinins play a physiological role In dogs enhanced activity of intrarenal kinins following kininase II inhibition was associated with renal vasodilatation diuresis and natriuresis (14) Rats treated with antibradykinin serum showed a reduced ability to excrete an intravenous saline load (11)

The validity of the equation presented by Sealey et al (16) as applied to individual patients was recently questioned by Maxwell et al (13) It is clear that individual variations in the relationship between PRA in renal veins and in peripheral vein are sizeable but the individual range for this relationship in essential hypertension was narrower in our study (98–158%) (8) than in earlier reports (13 16) We have assumed a plasma flow of 300 ml/min from the kidney with a non stenotic artery and also

Table II *Plasma renin activity (PRA ng/ml 3 h) renal plasma flow (RPF ml/min) renal venous kinins (ng/ml) and kinin output ratio (ng/min)*

	Pat 1	Pat 2	Pat 3	Pat 4
PRA_P	4 92	3 62	1 72	9 21
PRA_{NS}	5 38	3 65	1 76	8 97
PRA_S	11 20	9 42	5 48	29 45
PRA_S/PRA_P (%)	228	260	319	320
PRA_{NS}/PRA_P (%)	109	101	102	97
Assumed RPF_{NS}	300	300	300	300
Estimated RPF_S	141	112	82	82
RPF_{NS}/RPF_S	2 13	2 68	3 66	3 66
Kinin conc $_{NS}$	1 14	0 44	0 66	0 23
Kinin conc $_S$	0 43	0 44	0 37	0 32
Kinin conc $_{NS}$/ Kinin conc $_S$	2 65	1 00	1 78	0 72
Kinin $output_{NS}$/ Kinin $output_S$ (based on RPF ratio)	5 6	2 7	6 5	2 6

S=vein from kidney with artery stenosis NS=vein from kidney with non-stenotic artery P=peripheral vein

from each kidney in the patients with essential hypertension (16 17) This may be an overestimation but if a value of 250 ml/min is used instead the estimated kinin output ratios are not affected

Our results support the concept that a reduction of the renal artery pressure causing an increase in renin release is associated with a decrease in renal kallikrein release This diminished release of renal kallikrein may be the reason for the low diuresis and natriuresis regularly found in kidneys with artery stenosis

ACKNOWLEDGEMENTS

The studies were supported by grants from the Swedish National Association against Heart and Chest Diseases and the Medical Faculty of the University of Lund

REFERENCES

1 Adetuyibi A & Mills I H Relation between urinary kallikrein and renal function hypertension and excretion of sodium and water in man Lancet 2 203 1972

2 Barraclough M A & Mills I H Effect of bradykinin on renal function Clin Sci 28 69 1965

3 Bevan D R Macfarlade N A A & Mills I H The dependence of urinary kallikrein excretion on renal artery pressure J Physiol 241 34P 1974

4 Brown J J Owen K Peart W S Robertson J I S & Sutton D The diagnosis and treatment of renal artery stenosis Br Med J 2 327 1960

5 Haber E Koerner T Page L B Kliman B & Purnode A Application of a radioimmunoassay for angiotensin I to the physiologic measurements of plasma renin activity in normal human subjects J Clin Endocrinol 29 1349 1969

6 Hulthén U L Unpublished observation

7 Hulthén U L & Borge T Determination of bradykinin in blood by a sensitive radioimmunoassay Scand J Clin Lab Invest 36 833 1976

8 Hulthén U L Lecerof H & Hokfelt B To be published

9 Keiser H R Andrews M J Jr Guyton R A Margolius H S & Pisano J J Urinary kallikrein in dogs with constriction of one renal artery Proc Soc Exp Biol Med 151 53 1976

10 Margolius H S Geller R Pisano J J & Sjoerdsma A Altered urinary kallikrein excretion in human hypertension Lancet 2 1063 1971

11 Marin-Grez M The influence of antibodies against bradykinin on isotonic saline diuresis in the rat Pflugers Arch 350 231 1974

12 Marin-Grez M Cottone P & Carretero O A Evidence for an involvement of kinins in regulation of sodium excretion Am J Physiol 223 794 1972

13 Maxwell M H Marks L S Varady P D Lupu A N & Kaufman J J Renal vein renin in essential hypertension J Lab Clin Med 86 901 1975

14 Nasjletti A Colina Chourio J & McGiff J C Disappearance of bradykinin in the renal circulation of dogs Circ Res 37 59 1975

15 Nustad K The relationship between kidney and urinary kininogenase Br J Pharmacol 39 73 1970

16 Sealey J E Buhler F R Laragh J H & Vaughan E D Jr The physiology of renin secretion in essential hypertension Am J Med 55 391 1973

17 Vaughan E D Jr Buhler F R Laragh J H Sealey J E Baer L & Bard R H Renovascular hypertension Renin measurements to indicate hypersecretion and contralateral suppression estimate renal plasma flow and score for surgical curability Am J Med 55 402 1973

18 Webster M E & Pierce J V The nature of the kallidins released from human plasma by kallikreins and other enzymes Ann NY Acad Sci 104 91 1963

Table I *Essential clinical data on the patients*

	Pat 1	Pat 2	Pat 3	Pat 4
Age (y) and sex	20 ♀	32 ♀	62 ♀	42 ♂
Renal angiographic findings	Left artery stenosis min diam 1 0–1 5 mm post stenotic dilatation collateral circulation	Right artery fibromuscular dysplasia min diam 1 5 mm	Left artery stenosis min diam 1–2 mm poststenotic dilatation	Left artery stenosis min diam 1–2 mm poststenotic dilatation
Kidney size (cm)				
Right	14 5×6	12 5×6 5	15×7	16×6
Left	14×6	13 5×6 5	10×5	13×6
Serum creatinine (μM/l)	69	80	98	102
Creatinine clearance (ml/min 1 73 m²)	158	162	85	90
Supine BP (mmHg)				
Preoperative	160/110	180/110	200/110	180/120
Postoperative	125/80	130/85	160/85	140/90

normal renal function (8) Assuming a plasma flow of 300 ml/min from the kidney with a non stenotic artery the ratio of venous output of kinins between the kidneys could be calculated

RESULTS

All patients showed suppression of renin release from the kidney with a non stenotic artery PRA in the renal vein being 98–109% of the values found in peripheral vein In the vein from the kidney with artery stenosis PRA was 228–320% compared with peripheral vein (Table II) These findings indicate a hemodynamic significance of the renal artery stenosis (17)

Plasma flow from the kidney with artery stenosis estimated as described above ranged from 82 to 141 ml/min The ratio of venous output of kinins between the kidney with a non stenotic artery and the one with artery stenosis was 2 6–6 5 (Table II) Thus the output of kinins was significantly lower from the kidney with artery stenosis

DISCUSSION

The finding of a reduced venous output of kinins from the kidney with artery stenosis is in accordance with results concerning urinary kallikrein excretion in dogs Bevan et al (3) reduced renal artery pressure by a constricting clamp and found a highly positive correlation between urinary kallikrein excretion and renal artery pressure Keiser et al (9) found that urinary kallikrein excretion from the kidney with a ligated artery was significantly lower than from the contralateral kidney

There is good evidence that renal venous output of kinins reflects renal kallikrein activity During infusion of bradykinin into a renal artery in dogs the average amount of bradykinin recovered in the renal vein was less than 15% (14) In intact dogs acute intragastric saline loading increased the concentration of kinins in the inferior caval vein cephalic to the renal veins as well as the urinary excretion of kallikrein whereas saline loading in nephrectomized animals if anything slightly decreased the caval concentration of kinins (1?)

Several studies support the view that intrarenal kinins play a physiological role In dogs enhanced activity of intrarenal kinins following kininase II inhibition was associated with renal vasodilatation diuresis and natriuresis (14) Rats treated with antibradykinin serum showed a reduced ability to excrete an intravenous saline load (11)

The validity of the equation presented by Sealey et al (16) as applied to individual patients was recently questioned by Maxwell et al (13) It is clear that individual variations in the relationship between PRA in renal veins and in peripheral vein are sizeable but the individual range for this relationship in essential hypertension was narrower in our study (98–158%) (8) than in earlier reports (1? 16) We have assumed a plasma flow of 300 ml/min from the kidney with a non stenotic artery and also

Acta Med Scand 202 193–195 1977

The Concentration of Cadmium in Renal Tissue from Smokers and Non-Smokers

Karen Østergaard

From the Department of Pathology Hvidovre Hospital and the Institute of Pathology Sundby Hospital Copenhagen Denmark

ABSTRACT **The concentration of cadmium is determined by atomic absorption spectrophotometry of renal tissue excised at autopsy Tissue from 19 non smokers and 42 smokers of either sex in the age group 45–65 years has been analysed The concentration of cadmium in kidneys from 16 subjects who had been smoking pipes, cigars or cheroots did not differ significantly from the concentration in non smokers and cigarette smokers On the other hand, in both normotensive and hypertensive individuals, the concentration of cadmium in kidneys from cigarette smokers was twice as high as in kidneys from non smokers**

By now the harmful effects of tobacco smoking in particular of cigarette smoking on health are generally accepted and well documented Such effects include partly the direct action on the respiratory tract in the form of bronchitis emphysema and lung cancer partly the more indirect actions on other organs such as the arteries and the heart in the case of pregnant women also the action on the foetus Tobacco smoking intensifies the incidence of cancer involving the pancreas the bladder and other organs (1 4) Furthermore it has been observed that smoking may potentiate the action of other carcinogenic substances For instance among workers in the asbestos industry the incidence of lung cancer has been found to be 60 times higher among smokers than among non smokers (12)

It has been indicated in several investigations (2 6 7) that still another risk is involved namely that tobacco smoking results in an increased intake and accumulation of cadmium in the organism (2 6 7)

Cadmium is present as a trace element in soil and in several types of rock it is very closely allied to zinc with which it has certain chemical properties in common Cadmium may be taken up by plants and thus be incorporated in food its absorption from the gastrointestinal tract however is negligible (6%) Accordingly the cadmium burden is rather insignificant in populations in non industrialized areas The cadmium burden in the population will increase parallel with industrial development Cadmium may be inhaled in the form of dust or vapour in connection with the mining and use of zinc (objects of zinc brass galvanization) and of cadmium (cadmiation cadmium rich pigments) and also in connection with work using cadmium rich solders Owing to the appreciable absorption from the lungs (25–50%) it represents an essential risk to those thus exposed (1) The general population is exposed to cadmium from other sources as well for instance from cadmium rich kitchen utensils and chinaware from water mains and from percolators etc where cadmium from solderings may be dissolved Finally tobacco is a further source

Absorbed cadmium accumulates mainly in the kidneys from which it is excreted at a low rate Analyses of renal tissue have shown that the concentration of cadmium increases from the time of birth when cadmium is almost absent and up to the age of about 40–50 years when the concentration is approximately 2200 μg/g of ash The concentration of cadmium is seen to decrease again in older age groups (10)

Requests for reprints to Dr K Østergaard Patologisk Anatomisk Institut Rigshospitalet DK 2100 Copenhagen Ø Denmark

Table I. Age and sex distribution and cadmium concentration in the subjects investigated

	N	Mean age (y)	Cadmium concentration in whole kidney (µg/g ash)				
			Mean	Range	Geometric mean		
					All subjects	Normotensive	Hypertensive[b]
Non smokers	19	60.6	1 106	347–2 466	953	1 269 (n=6)	747 (n=11)
Males	10	62.5					
Females	9	58.4					
Smokers	42	57.7					
Males	37	57.5					
Females	5	59.0					
Pipe–cigar–cheroot	16	58.2	1 817	328–5 050	1 494		
<20 cigarettes/d	11	57.9	2 415	947–3 821	2 242 }	2 855 (n=10)	1 643 (n=9)
≥20 cigarettes/d	12	56.8	2 778	961–7 493	2 310 }		

[a] Three subjects were excluded during the study
[b] Systolic BP>160 mmHg or diastolic BP>95 mmHg

PATIENTS AND METHODS

The concentration of cadmium in kidneys has been determined in 61 Danish subjects in the age group 45–65 years whose smoking habits were on record. The distribution according to sex and age of subjects appears from Table I.

Wedge shaped specimens of renal tissue, weight 1–2 g, were excised with a stainless knife at autopsies in the period Jan. 1974 – Jan. 1975 and used for the investigation. Each tissue specimen comprised cortex as well as medulla extending up to the apex of the calyx. The tissue specimens were weighed and the samples were kept at [illegible] in polystyrene beakers provided with lids until analysis. The samples were dried at 100°C in silica crucibles for about 48 hours, whereupon they were ashed at 450°C for approximately 48 hours. The ashes were weighed and dissolved in 25 ml of 1 N HNO_3.

The solution was analysed for cadmium, using an atomic absorption spectrophotometer (Perkin Elmer model 300 provided with deuterium background corrector) without preceding extraction. A $CdCl_2$ solution served as standard. Two tissue specimens from each kidney were analysed.

RESULTS

The concentration of cadmium in kidneys from the 19 non smokers averaged 1 106 µg of cadmium/g of ash, in contrast to 2 268 µg/g in kidneys from the 42 smokers. The corresponding geometric means were 953 and 1 920 µg/g, respectively. On the basis of data obtained from the case records, the smokers could be classified into three groups: group 1 (pipe, cigar and cheroot smokers), group 2 (those who had smoked less than 20 cigarettes per day) and group 3 (those who had smoked more than 20 cigarettes per day). The length of time during which the subjects had been smokers was not specified. Three subjects were excluded from the series because they, according to the available information, had been in the habit of smoking cigarettes as well as pipes, cigars and cheroots. The results are recorded in Table I.

The statistical analysis was based on logarithmically converted values and showed that findings in the four groups differed significantly (F test, $p<0.001$). A comparison between groups showed a significant difference between non smokers and those who smoked <20 cigarettes per day (t test, $p<0.001$), while the median value of the group of subjects who smoked pipes, cigars and cheroots did not differ significantly either from values among non smokers or from median values among those who smoked less than 20 cigarettes per day ($p=0.9$ in either case).

DISCUSSION

It appears from several investigations (5, 11) that the BP and the concentration of cadmium in kidneys may be interrelated, so that a skew distribution over the two groups, normotensive and hypertensive individuals, may be responsible for the different concentrations observed. Data on BP were not available in two of the non smokers and in three of the cigarette smokers. It appears from Table I that the concentration of cadmium in kidneys from the remaining subjects is almost twice as high in cigarette smokers as in non smokers, whether normotensive or hypertensive. The difference is significant (t test, $p<0.001$).

It has been pointed out in several investigations from other countries that cigarettes are of importance as a source of cadmium. For instance Lewis et al. (6, 7) who analysed livers, kidneys and lungs obtained at autopsy of 172 American subjects found that the concentration of cadmium in kidneys from cigarette smokers was approximately twice as high as in kidneys from non-smokers and also that the concentration rises parallel with the number of cigarettes smoked. Hammer et al. (2) likewise found that the values of renal cadmium were twice as high in cigarette smokers as in non-smokers.

The content of cadmium in cigarettes has been analysed by Szadkowsky et al. (13) who in eight of the brands most commonly sold in W. Germany found that the amount of cadmium per cigarette averaged 1.4±0.22 µg. Nandi et al. (9) found in six of the brands sold in the USA an average content of 1.2 µg of cadmium per cigarette. Menden et al. (8) found that the amount of cadmium in American cigarettes ranged from 1.56 up to 1.96 µg. The combustion temperature in a cigarette is sufficiently high to bring about an evaporation of cadmium. Even so, Szadkowsky found merely 0.031 µg of cadmium in the gas phase of the smoke from one cigarette, the particles in smoke from one cigarette contain 0.47 µg of cadmium, in other words the main flow of smoke from one cigarette contains in total 0.178 µg of cadmium. Menden et al. (8) observed that the main flow of smoke from one cigarette contained from 0.1 up to 0.12 µg of cadmium. On the basis of findings in these and other investigations Friberg et al. (1) concluded that cigarette smokers inhale cadmium in quantities ranging between 0.1 and 0.2 µg per cigarette. As absorption of cadmium from the lungs is assumed to range at about 25–50%, the smoking of 20 cigarettes per day involves a daily absorption of 0.5–2 µg of cadmium (1). The daily absorption of cadmium from food is estimated to range between 0.5 and 1.5 µg and thus the smoking of 20 cigarettes may imply that the quantity of cadmium to be absorbed is doubled.

CONCLUSION

It is no easy matter to evaluate how the state of health of cigarette smokers will be influenced by this extra absorption of cadmium. Indeed renal damage will not be manifest until the concentration of cadmium approaches 12 000 µg/g of ash, a value hardly to be attained if the smoking of cigarettes is the exclusive source of extra cadmium. Other detrimental effects of cadmium which may be of interest in this context include damage to the liver, necrosis of the testis, hypertension and probably certain chronic lesions of the connective tissue (1, 4). So far, however, the concentrations of cadmium required for a development of these lesions cannot be predicted.

REFERENCES

1. Friberg L, Piscator M, Nordberg G F & Kjellstrom T. Cadmium in the environment. 2nd ed. CRC Press, Cleveland 1974.
2. Hammer D I, Calocci A V, Hasselblad V, Williams M E & Pinkerson C. Cadmium and lead in autopsy tissue. J Occup Med 15: 956, 1973.
3. Kallerup H E, Kierkegård Hansen G & Hansen J C. Exposure to cadmium as a possible precipitating factor in connective tissue disease. Ugeskr Laeger 138: 1396, 1976.
4. Kjeldsen K. Smoking and atherosclerosis. Munksgaard, Copenhagen 1969.
5. Lener J & Bibr B. Cadmium and hypertension. Lancet 1: 970, 1971.
6. Lewis G P, Coughlin L L, Jusko J W & Hartz S. Contribution of cigarette smoking to cadmium accumulation in man. Lancet 1: 291, 1972.
7. Lewis G P, Jusko W J & Coughlin L. Cadmium accumulation in man. Influence of smoking, occupation, alcoholic habit and disease. J Chron Dis 25: 717, 1972.
8. Menden E E, Elia V J, Michael L W & Petering H G. Distribution of cadmium and nickel of tobacco during cigarette smoking. Environ Sc Technol 6: 830, 1972.
9. Nandi M, Slone D, Jick H, Shapiro S & Lewis G P. Cadmium content of cigarettes. Lancet 2: 1329, 1969.
10. Schroeder H A & Balassa J J. Abnormal trace metals in man: Cadmium. J Chron Dis 14: 236, 1961.
11. Schroeder H A & Vinton W H. Hypertension induced in rats by small doses of cadmium. Am J Physiol 202: 515, 1962.
12. Selikoff I J. In: Clinical implications of air pollution research (ed. A J Finkel & W C Duel), pp. 49–66. Publishing Sciences Group, Acton, Mass. 1976.
13. Szadkowsky D, Schultze H, Schaller K H & Lehnert G. Zur ökologischen Bedeutung des Schwermetallgehaltes von Zigaretten. Arch Hyg (Berl) 153: 1, 1969.

Acta Med Scand 202 197–202 1977

External Carotid Pulse Recordings in Hypertrophic Obstructive Cardiomyopathy

Ola Alfred Nesje and Ivar Enge

From Medical Department B Laboratory of Cardiology and Roentgen Department University Hospital Rikshospitalet Oslo Norway

ABSTRACT External carotid pulse tracings were examined in 15 patients with hypertrophic obstructive cardiomyopathy (HOCM) the diagnosis being confirmed by catheterization of the left heart Of 12 patients with intraventricular gradients at rest, 9 had a typical bifid pulse with midsystolic dipping In one patient without a gradient at rest, midsystolic dipping occurred only in beats following extrasystoles (Brockenbrough phenomenon) The upstroke of the pulse wave was rapid in all the patients a finding that *distinguishes* them from patients with valvular aortic stenosis There was a correlation between the length of the left ventricular ejection time and the intraventricular gradient ($r=0\ 71$) but as more than half the patients had normal or shortened ejection times, the diagnosis of HOCM cannot be based on measurements of this parameter It is concluded that carotid pulse registrations are of considerable diagnostic value in patients suspected of having HOCM As the pulse changes are correlated to the degree of left ventricular outflow obstruction it is suggested that *repeated* pulse tracings may be used as a means for controlling the degree of obstruction once the diagnosis has been established in the individual patient

Soon after hypertrophic obstructive cardiomyopathy (HOCM) also called idiopathic hypertrophic subaortic stenosis or asymmetric septal hypertrophy (8) had been described as a clinical and pathological entity (2 14) attention was directed to the associated changes in the pulse contour (1)

By intra aortic flowmetry Hernandez et al (10) found that the left ventricular outflow is exceptionally rapid at the start of systolic ejection in patients with HOCM These patients eject about 80% of their stroke volume during the first half of the systole compared with 55–60% in normal persons When the muscular obstruction to ventricular outflow begins the orthograde flow in the aorta decelerates rapidly causing a fall in the aortic pressure This flow pattern gives rise to a bifid pulse waveform in the aorta and central arteries the pulse rises rapidly to a peak then dips sharply in midsystole and rises again in late systole Frank and Braunwald *(7) found this pulse contour in external tracings* from the carotid artery in 66% of 111 patients with HOCM

While it is well known that the left ventricular ejection time (LVET) is prolonged in valvular aortic stenosis the relationship between the degree of ventricular outflow obstruction and LVET is less clear in patients with HOCM Whereas some authors have found a good correlation between the intraventricular gradient and the LVET (17) such a relationship has not been confirmed by others (11)

In the present study we have examined the carotid pulse tracings from 15 patients with HOCM and evaluated three components of the pulse separately 1) configuration of pulse wave 2) rapidity of upstroke and 3) length of the LVET

PATIENTS AND METHODS

The study population consists of 4 women and 11 men *their ages ranging from 21 to 64 years* The diagnosis of HOCM was established in all patients by left heart catheterization An intraventricular gradient at rest was found in 12 patients and in 3 patients a gradient could be provoked by isoprenaline infusion (Table I) Angiography was performed in 14 patients and showed diffuse ventricular hypertrophy in all

A simultaneous registration of the external carotid

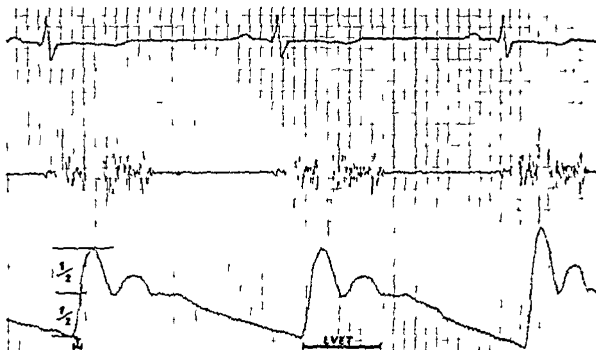


Fig 1 Recording from a patient with hypertrophic obstructive cardiomyopathy *Top* ECG *middle* high frequency PCG at left sternal border showing systolic murmur of ejection type *bottom* carotid pulse with midsystolic dipping *T*=*T* time *LVET*=left ventricular ejection time

pulse the PCG at the left sternal border and the ECG was made on a Mingograph 61 recorder as previously described (12) The paper speed was 100 *mm/sec* and the pulse tracings were made with a photoelectrical recorder (Hertheine Siemens) which has a time constant of 2 6 sec (Fig 1)

The rapidity of the pulse upstroke was measured in terms of the *T* time which is the time required for the pulse to attain one half of its peak height (5) The normal value for the *T* time is 20–46 msec (13)

The LVET was measured from the beginning of the pulse upstroke to the incisura caused by the closing of the

Table I *Intraventricular gradient and carotid pulse tracing*

Pat no	Sex	Age (y)	Rhythm	Intraventricular gradient (mmHg)		Carotid pulse tracing		
				At rest	On isoprenaline	Configuration	*T* time (msec)	ΔLVET (msec)
1	♂	48	SR	0	60	Normal	30	−46
2	♂	63	SR	0	64	Flattened	30	−39
3	♂	61	SR VES	0	90	Varying	25	−59
4	♂	50	SR	18	54	Flattened	20	−57
5	♂	48	AF	40		Varying		
6	♂	40	SR	42		Bifid	35	−37
7	♂	73	SR	45		Flattened	35	−28
8	♂	21	SR	48		Bifid	35	+36
9	♂	31	SR	62		Bifid	25	34
10	♀	23	SR	70		Bifid	20	−5
11	♂	48	SR	94		Bifid	25	+33
12	♀	64	SR	102		Bifid	20	+32
13	♂	55	SR	105		Bifid	30	0
14	♀	46	SR	120		Bifid	30	+1
15	♀	63	SR	165		Flattened	25	+25

SR=sinus rhythm AF=atrial fibrillation VES=ventricular extrasystoles

Fig 2 Record ng from a patient w th hypertrophic obstructive card omyopathy and atrial fibrillation Top and bottom trac ngs as in Fig 1 The two m ddle tracings show low frequency PCG (*above*) and high frequency PCG (*below*) After long RR intervals the midsystolic d ps deepen and the systolic murmurs grow louder

aort c valves The deviat on of the measured LVET from the expected LVET (ΔLVET) was calculated by subtract ing the former from the latter at the given heart rate using We ssler s formulae (15) *Accord ng to these formulae* the expected normal LVET for women is 418 msec—1 6 × heart rate and for men 413 msec—1 7 × heart rate

RESULTS

The results are presented in Table I It will be seen that of the 12 patients with an intraventricular gra d ent at rest 9 had typical bifid pulses with a sharp d pping in midsystole One of them (no 5) had atrial fibrillation and his pulse wave tracing showed a positive correlation between the depth of the midsystolic dips and the length of the preceding RR interval (Fig 2) In patients 4 7 and 14 whose gradients were 18 45 and 165 mmHg respectively the pulse showed abrupt flattening but no clear dip ping after reaching its peak height Patients 1 2 and 3 had no intraventricular gradient at rest and the pulse configuration was normal and rounded in pa tient 1 and flattened in patient 2 whereas in patient

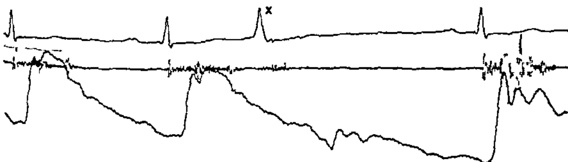


Fig 3 Effect of extrasystoles on the pulse contour in hypertroph c obstruct ve card omyopathy Tracings as in Fig 1 In the s nus beat following the premature beat (×) the carotid pulse shows d pp ng and the systolic murmur intens fes

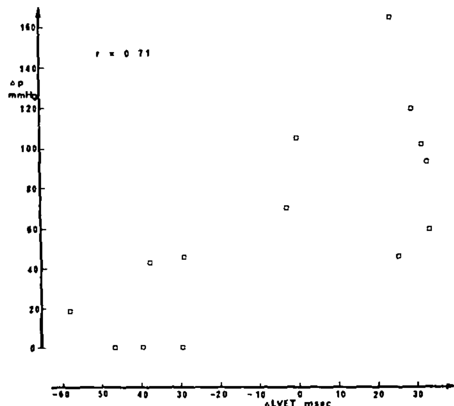


Fig 4 Intraventricular gradient at rest (Δ*p*) and deviation of left ventricular ejection time from the expected ejection time (Δ*LVET*) in 14 patients with sinus rhythm

3 the pulse was normal except in beats which were preceded by ventricular premature beats These postextrasystolic beats showed typical midsystolic dipping (Fig 3)

The *T* time was invariably normal in all patients ranging from 20 to 35 msec There was no correlation between the *T* time and the intraventricular gradient

Among the 14 patients in sinus rhythm the LVET was shortened in 6 unchanged or not changed by more than 5 msec in 3 and lengthened in 5 There was a positive correlation (r=0 71) between ΔLVET and the intraventricular gradient at rest (Fig 4) In patient 5 with atrial fibrillation the length of the LVET increased on gradual lengthening of the preceding RR interval and no absolute value for the LVET could be determined

DISCUSSION

Our results confirm that the majority of patients with HOCM have an abnormal pulse contour (6 7) The finding of a sharp midsystolic dipping in 9 of the 12 patients with pressure gradients at rest is of great diagnostic significance since such a pulse wave form is virtually pathognomonic for HOCM (13) Although the sudden flattening of the pulse wave in the other 3 patients with rest gradients is decidedly abnormal it is not diagnostic for HOCM

In patient 3 the pulse contour was influenced by premature beats and in patient 5 by the length of the preceding RR interval These pulse changes reflect a variation in the intraventricular obstruction caused by the arrhythmia The finding is characteristic for HOCM and has been called the Brockenbrough phenomenon

An extrasystole which fails to empty the left ventricle will increase the end-diastolic volume and by the Frank Starling mechanism this increased volume will lead to enhanced contractility in the next regular beat In patients with HOCM however an increase in contractility will also increase the obstruction to ventricular outflow causing a fall in the aortic pressure and a dipping in the carotid pulse (3) In atrial fibrillation the end-diastolic volume of the left ventricle depends on the length of the filling time A long diastole or RR interval increases the end-diastolic volume and thereby also contractility and outflow obstruction (9) It cannot be excluded however that an increase in electrical stimulation following extrasystoles in patients with ventricular

extrasystoles and long periods of rest in patients with atrial fibrillation may increase ventricular contractility (16)

The *T* time was normal in all patients and as this measurement reflects the rapid aortic flow prior to the onset of outflow obstruction this finding is not surprising The fact that the *T* time is normal in patients with HOCM is of clinical import n e when it comes to separating them from patie nt s th val vular aortic stenosis In this condition t r stroke of the pulse is slower than normal and me is usually prolonged (5 13)

The deviations of the LVET from ted normal value correlated fairly well h in traventricular gradient at rest patien ent or small gradients having shortened n es and patients with gradients above 45 mal or lengthened The distribution of the of the LVET on both sides of the ex al value however limits the diagnostic f LVET changes in the individual pat t dent that the finding of a shorten al LVET does not preclude the diagno M On the other hand changes in the ter pharmacological interventions for e tion of amyl nitrite may well be of di alue in the individual patient (4)

We found that all patients with an intr entr ular gradient above 42 mmHg at rest had pathological pulse waves Frank and Braunwald (7) reported a correlation between the occurrence of bifid pulse waves and the magnitude of the intraventricular gradient patients without gradients at rest usually having normal pulse waves These findings suggest that a normalization of the pulse contour would denote a reduction of the intraventricular gradient in a patient with HOCM

We further found a correlation between the intraventricular gradient and the length of the LVET a finding that corresponds with the results of Wigle et al (17) Although the length of the LVET is of limited use for the diagnosis of HOCM in the individual patient it seems that once the diagnosis has been established a shortening of a previously lengthened LVET would indicate a reduction of the intraventricular gradient in the patient

While the degree of outflow obstruction can be controlled by recordings of the carotid pulse the clinical condition of the patients may not be predicted from the pulse changes Follow up studies of patients with HOCM have shown that symptoms and prognosis tend to be related not to the degree of outflow obstruction but to the height of the end diastolic pressure of the left ventricle (8) The high end diastolic pressure is caused by the low distensibility of the stiff and hypertrophic myocardium which resists ventricular filling during diastole Although the diastolic component of the abnormal ventricular function in HOCM is not reflected in changes in the carotid pulse the systolic component of the disease—the obstruction to outflow—does change the pulse in a characteristic way

Our conclusion is that carotid pulse [illegible] of clinical use both as a diagnostic mean [illegible] suspect of having HOCM and as a [illegible] trolling the degree of outflow obstruction [illegible] known to have the disease

REFEREN

1 Brachfeld N & Gorlin R [illegible] vised concept of the disease
2 Brock R Functional obstru[illegible] Guys Hosp Rep 106 221 19[illegible]
3 Brockenbrough E C Brau[illegible] G A hemodynamic techn[illegible] hypertrophic subaortic sten[illegible] 1961
4 Cohn K E Flamm M D [illegible] nitrite inhalation as a screen[illegible] subaortic stenosis Am J Car[illegible]
5 Duchosal P W Ferrero C [illegible] ta E Advance in the cl[illegible] stenosis by arterial pulse rec[illegible] Heart J 51 861 1956
6 Epstein S E In Asymme[illegible] NIH Conference (moderator[illegible] tern Med 81 650 1974
7 Frank S & Braunwald E [illegible] subaortic stenosis Circul[illegible]
8 Goodwin J F [illegible] IHSS [illegible] unity Am Heart J 89 26[illegible]
9 Hancock E W & Eldr[illegible] stenosis Am J Cardiol 1[illegible]
10 Hernandez R R Gre[illegible] W Pressure flow studie[illegible] stenosis J Clin Invest 4[illegible]
11 Ibrahim M Silie M [illegible] R Systolic time interv[illegible] and idiopathic hyper[illegible] Heart J 35 276 1973
12 Nesje O A Måling [illegible] ublodig metode til [illegible] funksjon Tidsskr N[illegible]
13 Tavel M E Clinic[illegible] nal pulse recording [illegible] Chicago 1972

14 Teare D Asymmetrical hypertrophy of the heart in young adults Br Heart J 20 1 1958

15 Weissler A M Harris W S & Schoenfeld C D Systolic time intervals in heart failure in man Circulation 37 149 1968

16 Van der Werf T van Poelgeest R [illegible] H H & Meyler F L Postextrasystolic [illegible] in man Eur J Cardiol (Suppl) 4 131 1976

17 Wigle E D Auger P & Marquis Y [illegible] subaortic stenosis Circulation 36 36 196[illegible]

Acta Med Scand 202 203–212 1977

Respiratory Distress Syndrome and Thrombotic, Non-Bacterial Endocarditis after Amitriptyline Overdose

Folke D Lindström Olof Flodmark and Bertil Gustafsson

From the Departments of Medicine Radiology I and Pathology II University Hospital Linköping Sweden

ABSTRACT Three patients with documented or suspected amitriptyline poisoning developed a uniform clinical picture with progressive respiratory insufficiency thrombocytopenia and evidence of embolization Post mortem examination revealed equally uniform changes thrombotic non bacterial endocarditis (TNBE) with multiple embolization and marked pulmonary stiffness with extensive invasion of alveoli with connective tissue The lung pathology and clinical picture of progressive respiratory insufficiency are in agreement with the respiratory distress syndrome (RDS) in the adult The simultaneous occurrence of RDS and TNBE suggests a similar pathogenesis *Prophylactic measures are described*

The use of tricyclic antidepressive drugs has increased since they were introduced in the late 50s Intoxications due to an overdose of these drugs have risen parallel with this and are now more common than barbiturate intoxication in Sweden The symptoms are therefore well known and include coma cramps hypotension and depressed respiratory activity The most dreaded effects of an overdose are various potentially fatal irregularities of the heart action Pulmonary disease secondary to tricyclic drug intoxication has been described in two instances (20 28) one of which was fatal (20) In spite of the serious character of tricyclic overdose mortality has been low in most published studies (21)

Reprint requests to F D Lindström M D Department of Medicine University Hospital S 58185 Linköping Sweden

During 1975 we observed two fatal cases of amitriptyline overdose at the University Hospital Linköping Sweden Both patients seemed to succumb from a severe respiratory insufficiency accompanied by widespread alveolar and interstitial infiltrates At autopsy both cases exhibited soft friable vegetations on the heart valves plus embolic phenomena consistent with thrombotic non bacterial endocarditis (TNBE) as well as heavy stiff lungs

In this presentation we include a third case with an almost identical sequence of events and autopsy findings Our probable diagnosis in this case was tricyclic drug intoxication—on the basis of the history and the similarity to the other two cases—but we lack definite knowledge about the precipitating event With this study we would like to focus attention on an uncommon complication to amitriptyline overdose

STUDY POPULATION

Case I

Female 30 years previously well Admitted after taking amitriptyline tablets total dose 6 3 g and an unknown quantity of Klopoxide tablets Serum and urine levels of amitryptyline on admission were 15 and 5 μmol/l respectively The patient was deeply unconscious when first seen BP 70/60 mmHg Soon thereafter she became pulseless ECG showed nodal rhythm with bundle branch block pronounced widening and deformity of QRS complexes After a few seconds this transformed into ventricular tachycardia and fibrillation The patient was defibrillated and then developed asystole After resuscitation sinus rhythm but AV block I and moderate QRS widening During this episode the patient was given 2 mg



Fig 1 Marked infiltration of both lungs with air bronchogram (patient 2)

of physostigmine salicylate i v Subsequently she was connected to a respirator and during days 2–9 gradually regained consciousness meanwhile however bilateral pulmonary alveolar infiltrates visible on chest X ray developed and then partly disappeared During the first days she received high concentrations of oxygen After day 10 however bilateral pulmonary infiltrates rapidly developed and at the same time the PaO_2 level fell (55–60 mmHg) in spite of 100% oxygen from the respirator There was a progressive lowering of the thrombocyte el from 154000 on day 1 to 12000/mm³ on day 14 n the patient died She had normal fibrinogen level ring the entire period no evidence of fibrinolysis leparin was given on days 4 10 and low molecular weight dextran during her last 74 hours PaO_2 values remained subnormal despite 100% oxygen and the patient became gradually more cyanotic

At autopsy verrucose vegetations were noted on aortic mitral and tricuspid valves thrombotic masses in various organs an embolus in the left medial cerebral artery and infarctions in heart kidneys spleen and lung The lungs were markedly consolidated and the pulmonary artery had grey red thrombotic masses in its peripheral branches We could not establish whether these thrombi were emboli from venous thrombosis in the vein plexus of the uterus from vegetations on the tricuspid valve or if they were thrombi generated in situ

Case 2

Female 45 years Poliomyelitis in 1953 without sequelae otherwise previously well Admitted after an overdose of amitriptyline 750 mg When first seen she was stuporous but could be aroused systolic BP 60 mmHg ECG and chest X ray were normal (Nortriptyline/plasma 60 μg/ml on day 2) During the first two days the patient improved and regained normal consciousness while being ventilated by a respirator via an endotracheal tube From day 3 to day 7 progressive bilateral pulmonary changes were evident on chest X ray as increasingly dense alveolar infiltrates (Fig 1) Concurrently there was a d e PaO₂ level requiring increasing concentrations of oxygen from the respirator During this time there was a d in thrombocyte level from 102000 to 51000 mm³ On the 7th day there was an episode of asystole but the heart resumed normal activity after a brief resuscitation On days 11–12 ECG changes were noted and an anterior wall myocardial infarction and the diagnosis was confirmed by enzyme changes The patient died from asystole on day 13 There was no change in the fibrinogen level and no evidence of fibrinolysis Heparin was given from day 2 through day 1?

At autopsy verrucose changes (each 10 mm long were noted on the mitral valves There was an embolus in the descending branch of the left coronary artery and an extensive myocardial infarction including most of the wall of the left ventricle There were multiple infarctions in the spleen and both kidneys Both lungs were heavy and very stiff

Case 3

Female 17 years previously well On day 1 she had reportedly taken an unknown amount of nerve tablets and some beer become sleepy and fallen thereby h g her head slightly She was observed at another hospital overnight and was discharged since no abnormality had been noted On the following day she gradually became unconscious developed irregular twitchings in the extremities and slight cyanosis On admission she was cyanotic hyperventilating comatose and had cramps On auscultation of her lungs rales were heard suggesting obstruction of the airways by secretion Neurological examination lumbar puncture and bilateral carotid angiogram revealed normal conditions and tended to exclude intracranial bleeding and focal CNS damage Drug intoxication was considered the most likely explanation and the patient was intubated and connected to a ventilator Chest X ray on admission showed centrally located alveolar infiltrates interpreted as pulmonary edema The patient was given 100% oxygen from the respirator but despite *this had a PaO_2 of only 70 mmHg* However oxygen concentration in inspired air could be decreased on the following day Improved wakefulness on days 5 and 6 Elevated enzyme levels were noted (GOT GPT LDH and CPK peak values 1340 1280 5750 and 1000 U respectively)

Gradual decrease in Hb values occurred w h a low of 8 1 g/100 ml unexplained by bleeding or hemolysis and as a consequence the patient was given blood transfusion Thrombocytopenia (35000–19000 mm³) was noted during the entire hospital stay After an initial improvement chest X ray showed progressive worsening with extensive alveolar and finally interstitial infiltrates and air bronchogram Concurrently increased oxygen concentrations were required to keep PaO_2 on an adequate level On day 10 there was evidence of obstruction of the arterial flow to the right hand cyanosis and decreased temperature of the fingers and disappearance of the radial pulse Subsequently progressive deterioration with respiratory insufficiency poor circulation and death on day 14 followed Heparin was instituted on day 9



Fig 2 Thrombot c non bacterial endocard t s on mitral valves Verrucose vegetat ons are partly covered by clotted blood

At autopsy (performed by B Gard n and L Rammer Department of Forensic Med c ne Un vers ty Hosp tal Linkop ng) thrombotic vegetat ons were found on m tral and aort c valves Both lungs were heavy and markedly consol dated An embolus (or thrombus) was found in the right rad al artery Several areas of infarction were noted n the spleen heart and kidneys

RESULTS

In several important respects there was a remark able similarity between the three cases both in the way compl cations developed and in autopsy find ngs The common traits are therefore considered together below

Essentially the same treatment schedule was used for all three pat ents Respirator treatment was used during the entire hospital stay with varying oxygen concentrations All three patients received 100% oxygen during part of their sickness Positive e d-expiratory pressure was used for part of the t me Parenteral nutrition was given crystalloids mostly—many t mes in large quant t es—and col lo ds in the form of albumin and lip ds (Intra l p d[2]) Antibiotics (penicillin and ampicillin) were given and corticoids in moderate dosage

Multiple infarctions were found in the heart spleen and kidneys Pulmonary X ray changes in all pat ents showed a s m lar course At first there were relat vely l mited alveolar infiltrates that partly dis appeared—only to be followed by progressive severe alveolar and interstitial infiltrates giving the impression of white lung with air bronchogram Very striking and paradoxical was the init al impro vement with increased wakefullness and part al re gression of pulmonary disease followed by pro gressive and relentless respiratory insufficiency leading to death All three cases developed throm bocytopenia without bleeding tendency concur rently with the appearance of progressive pulmo nary changes on X ray The duration of disease was 1?–14 days

Post mortem examination

The autopsy f ndings were almost identical both on gross and on microscopic exam nation and are therefore described jointly in the following The valvular vegetations had the characteristics of TNBE (Fig ?) (? ?9) There were no signs of bac terial invasion or accumulation of inflammatory cells in the thrombot c vegetations valves or adja cent myocard um (Fig 3) Culture of thrombot c material from the valves obta ned at autopsy d d not produce bacterial growth

The normal pulmonary arch tecture was difficult to recognize in many places owing to widespread invas on of alveoli and alveolar ducts by connective tissue abundant in collagen Alveolar septa were widened with increased amounts of connective tis sue (Fig 4) Hyaline membranes were observed in two pat ents (Fig 5)

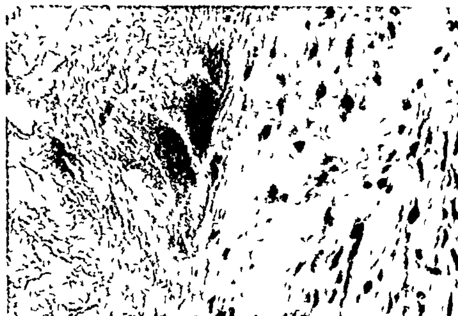


Fig 3 Microscopy of section of mitral valve (right) with adhering thrombotic vegetation (left) Note complete absence of inflammatory cells in both valve and thrombus Hematoxylin-eosin ×156

Using special fat stain (oil red O) abundant fat was noted in the lungs most of it inside macrophages situated in the connective tissue and alveoli (Fig 6*a* and *b*)

Large amounts of thrombi were noted in several organs located in vessels of intermediate size (mostly arteries) but not in capillaries (Fig 7) It was not possible to establish whether the thrombi were formed locally in the vessel or if they constituted emboli from vegetations on the heart valves We consider it significant however that the only patient having thrombi peripherally in the pulmonary artery was also the only one with endocarditic changes on the tricuspid valves

DISCUSSION

The autopsy findings clearly indicate that the patients had TNBE Characteristic are friable white yellow vegetations consisting of fibrin and corpuscular elements from the blood that may develop on all four heart valves It is distinguished from

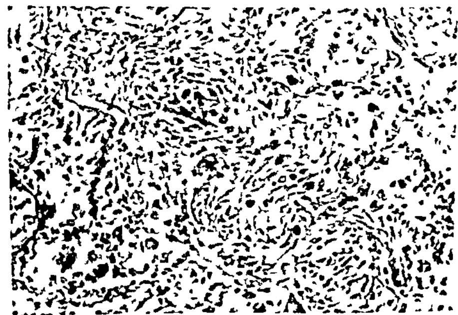
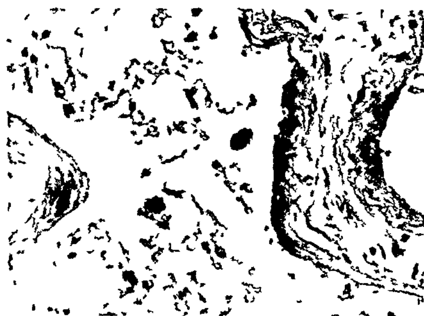


Fig 4 Pulmonary alveolar duct completely invaded by connective tissue In adjacent alveoli macrophages and desquamated epithelial cells are seen as well as incipient connective tissue invasion Hematoxylin-van Gieson ×156



F g 5 Lung show ng al eolar walls covered by hyal ne membranes *In er alveolar* septa are widened and contain ncreased quant ty of *connect ve* tissue *Period ac d* Sch ff ×163

bacterial *endocard tis* through m croscop c exam nat on n bacterial endocard tis bacteria are found n the thrombus and there is also an *abundance* of inflammatory cells in the underlying valve *itself* wh le *these phenomena* are not seen in TNBE *The* friable valvular vegetations *easily break loose* and are carried away by the bloodstream *caus ng* embol c les ons mainly in the heart *spleen and k d* neys In the case of *right sided* valvular vegeta tions emboli go to the lungs

TNBE occurs mainly in pat ents with *malignant* tumors and other long standing *severe and de* bil tat ng d sease (2 29) *The* age of patients af fected *varies* between reports but the *d sease* seems *to be* very uncommon in *young ind v duals* TNBE as a complicat on to drug *intoxication* has not been described prev ously The et ology of the disease is unknown but disseminated intravascular coagulat *on* and increased tendency *to platelet ag* gregat on have been discussed as *being involved in* the pathogenesis

The w despread progressive pulmonary d sease seen *n these* three *pat ents has* previously been described in a case *of fatal amitriptyline* intoxica tion (20) At autopsy *this* case showed pulmonary changes very sim lar to those *in our three cases* Nol endocard tis was not ment oned *in the report* The authors thought *that the lung* d sease was secondary to a toxic *effect of the* tricycl c drug on the surfactant *system of the lung* This interpreta tion was based on the observation of Hucker and Porter *(15)* that amitriptyl ne is elim nated rap dly from the blood and accumulates in lungs *brain* heart *where the concentration* may be up to 180 t *mes higher th* in in plasma

The lung d sease we observed in the three pa t ents seems to f t very well with a d sease ent ty commonly referred to as respiratory *distress syn drome (RDS)* (4 5) but which is also known under approximately 30 synonyms (wh te lung syndrome *fat* embol sm shock lung st ff lung DaNang lung progressive pulmonary *consolidation etc*) *RDS* is *characterized* by *rapidly* progressive lung infil *trates on X* ray lowering of PaO_2 thrombocyto penia and at autopsy heavy st ff lungs these features were all *found in* our pat ents M croscop c *examinat on* of lungs from patients dying from RDS typ cally shows hyal ne membranes which result in f brous obliterat on of respiratory bronchioles and alveolar ducts if survival is suff ciently long (23)

RDS may develop in conjunct on with a variety of d sease *states* such as severe *trauma burn* sept cemia shock mult ple transfusions *Th s sup* ports a mult ple f ctor hypothes s of *the pathogene* sis *of RDS* *The* un *form* cl n cal appearance of the syndrome on the other hand indicates a non spec fic response of lung tissue to a multitude of insults—a final common pathway Initiat ng fac tors accord ng to Safar et al (25) include *hypoper* fusion flu d overload *hyperoxia aspirat on* Ash

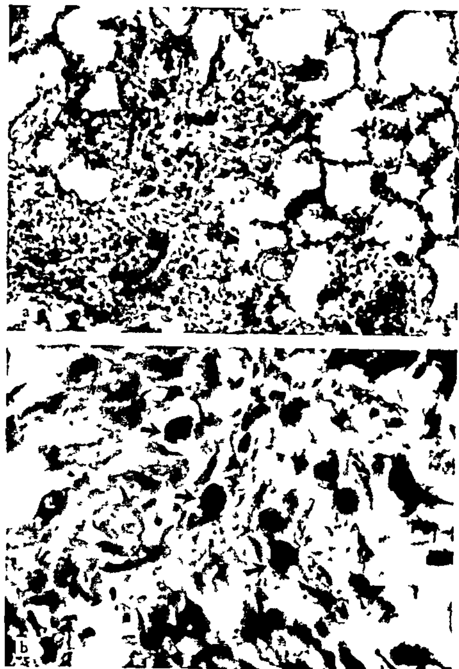


Fig 6 (a) Low power view of fat stained lung showing partial replacement of alveoli with connective tissue and widened interalveolar septa. Dark spots indicate lipid filled macrophages. Oil red O ×64. (b) Pulmonary alveoli invaded by connective tissue. Fat stain showing several lipid filled macrophages (arrows). Oil red O ×256

baugh et al (1) reported 12 cases of RDS two of which were precipitated by drug ingestion. They felt that pathogenesis in RDS as well as in RDS of the newborn may be related to a decrease in the surfactant agent but that other factors may contribute such as fluid overload and hypotension. Experimental work and clinical studies by Saldeen (26) have indicated that RDS is a microembolic phenomenon and that microthrombi develop in the periphery and then embolize to lung capillaries where they cause mechanical obstruction. As a consequence capillary damage develops causing increased permeability leakage of protein rich exudate into the alveoli and further lung damage.

The clinical course in our patients with progressive lung changes developing together with progressive thrombocytopenia—and also the autopsy findings with an abundance of thrombi in vessels of intermediate size, multiple organ infarcts and TNBL—strongly suggests that the disease manifestations are caused by thrombocyte aggregation formation of thrombi in the periphery and subse-

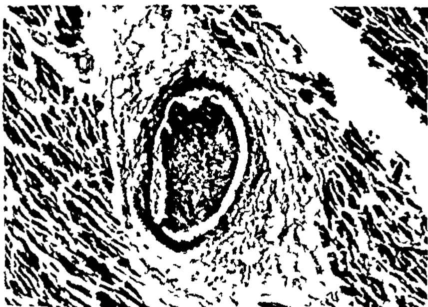


Fig 7 Section of myocardium showing a blood vessel containing thrombotic material PTAH gives a blue (dark) stain to myocardium and fibrin Note scarcity of fibrin in thrombotic material PTAH ×51

quent embolization The formation of thrombotic vegetations on a rapidly moving structure like the heart valve indicates a very marked tendency towards thrombocyte aggregation

In Fig 8 we have listed factors that may be of pathogenetic significance *Aggregation of platelets* preceded by a state of increased platelet adhesiveness is precipitated by release of thromboplastin which follows tissue damage (9) Also the platelets tend to adhere to damaged tissue such as subendothelial or *collagen structures (3) The type of* tissue damage is unspecific and in our patients different kinds of lung damage may have occurred anemia hypoperfusion aspiration and hypothetical toxic effect of amitriptyline accumulation (20) The aggregation of platelets *in itself causes release of* factors like adenosinediphosphate and serotonin (14) which induces further platelet aggregation It is possible that high concentrations of amitriptyline in the lungs may have had a toxic effect on cells

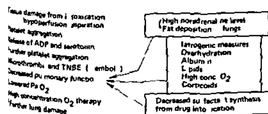


Fig 8 Hypothetical factors in pathogenesis of respiratory distress syndrome—thrombotic non bacterial endocarditis

responsible for surfactant synthesis Lack of surfactant causes alveolar instability atelectasis and transudation of protein rich fluid that may eventually lead to formation of connective tissue (26)

Many of the factors mentioned above participate in the *normal hemostatic sequence After severe or* multifactorial tissue damage there may be excessive stimulation of these mechanisms giving rise to thromboembolism with deleterious effect Sepsis shock trauma etc may also inhibit the *fibrinolytic system which normally tends to balance or* counteract effects of excessive coagulation On the basis of what we have observed in the three cases it is reasonable to assume that the endocarditis as well as the initial pulmonary changes are caused by *platelet aggregation Intravascular thrombi* observed in other organs may have been caused by the same mechanism but it is more likely that they constitute embolic phenomena

The striking accumulation of fat in the lung alveoli has led us to consider an alternative pathogenesis of the lung disease The fat deposits could have been caused by the tricyclic drug via an increased level of catecholamine in the circulation Tricyclic drugs are very active inhibitors of tissue uptake and inactivation of noradrenaline (17 18) This effect most likely tends to elevate or maintain a noradrenaline level that is probably already elevated by the intoxication in itself Experimental work (8) has shown that infusion of noradrenaline is followed by elevated plasma levels of free fatty

acids and triglycerides and also deposition of fat droplets in e g lung alveoli (macrophages) Furthermore a high dose intake in rats of the tricyclic drug iprindole (Prandole®) causes widespread degenerative changes in the lungs and numerous lipid inclusions in the epithelial cells there (27) Thus the excessive fat accumulation in the lungs observed in our patients may be mediated through elevated noradrenaline levels in the circulation and/or be a direct result of the tricyclic drug The possibility that the fat deposits in the lungs are derived from lipids given i v to all three patients cannot be excluded Pulmonary fat deposition probably interferes with gas exchange and may also encourage further platelet aggregation

Since lung damage and TNBE evidently are uncommon complications to tricyclic drug intoxication and since we have observed two or three cases during a short period in our hospital it must be asked whether there is something in our management of patients that favours the emergence of these fatal complications Obviously the prognosis was very poor in the patient who had ingested 6 g amitriptyline but in the other two patients we encountered exactly the same complication even though the dose was relatively low or a tricyclic drug intake was only suspected Could iatrogenic measures be of importance? High concentrations of oxygen in inspired air rapidly lead to pulmonary fibrosis (24) causing even more disturbed gas exchange in all three cases 100% oxygen was given during several days

Overenthusiastic fluid treatment may lead to fluid overload in the lungs resulting in poor gas exchange (12) Large volumes of crystalloids were given to all patients and in patient 3 the first chest X ray showed evidence of pulmonary edema However no direct relationship can be established between fluid overload and RDS (13) Also it is difficult to conceive how fluid overload in the lungs as a single etiological factor could cause the very marked pulmonary fibrosis observed at autopsy in all three cases

The use of albumin in RDS may result in further lung damage increased capillary permeability in this disease may lead to escape of albumin into interstitial fluid where it could result in increased pulmonary edema (19)

The fat deposition in the lungs may have been endogenous or exogenous but there is no means of telling which Intravenous infusion of lipids (Intralipid®) does give an increase in platelet adhesiveness as measured with Hellem's whole blood method (10) but in the same study this is in erpr ... as an in vitro effect and the conclusion is that I... lipid® does not have any effect on the coag... system

RECOMMENDATIONS

On the basis of our experience from these cases we would like to recommend a few prophyla... measures to be taken in cases of severe tric... poisoning We have listed these recommenda... as follows

Avoid Excessive hydration Albumin—u... needed as plasma expander Lipids intravenous... Corticoids High concentration (>60%) oxyg... inspired air *Use* Positive end-expiratory pressu... As anticoagulant dextran (mol wt 70000) alo... or in combination with heparin Experimental trea... ment membrane oxygenator + pulmonary lavage

Too generous fluid therapy should be avoide... Indeed in a similar case with progressive pulmo... nary changes thrombocytopenia etc it has bee... possible to treat a young woman with tricyclic ove... dose to a happy outcome by adhering to very s... fluid restriction (personal communication from B Schildt D Cochran and G Hedenstierna Ser... fimerlasarettet Stockholm Sweden) That f... overload in this patient was not the only ca... was evident from an extremely low complian... which improved very slowly months after the in... episode

Albumin should be used only on very str... indications

High concentrations of oxygen in inspired a... (>60%) should be avoided since they may cause or aggravate lung damage

Intravenous administration of lipids should be avoided This may cause lung damage and there ... no need for a high calorie nutrient like lipids d... the relatively short time span of a drug int...

Patient 2 received prophylactic heparin trea... during most of her disease the other two were given heparin for only 6 and 5 days Heparin ... therefore not effectively prevent the widespread thromboembolism noted in all three cases Th... heparin is ineffective in preventing RDS has bee... shown by others (23) and there is experimental evidence (6) that heparin may induce ... platelet aggregation The post mortem exam...

gives us reason to believe that an important pathogenetic mechanism here is the formation of microthrombi. The initial stage in the formation of all thrombi is adhesion and aggregation of platelets on the vessel wall. Heparin has no effect on this stage.

The antithrombotic effect of dextran was discovered in 1959 and has been demonstrated repeatedly in several experimental and clinical studies (7-11). In fact, dextran with a molecular weight of 70000 (Macrodex®) is the most effective agent known for reducing platelet adhesiveness (22). Thus it is possible that treatment with dextran or dextran + heparin can more effectively prevent the formation of widespread microthrombi. For this reason we would like to suggest the use of dextran as a prophylactic measure in severe tricyclic intoxication. As both RDS and TNBE may be expressions of the same pathogenetic mechanism, the suggested prophylaxis for RDS should be suitable for TNBE as well.

Fibrinolytic activity is a normal property of vessel walls (16) and counteracts the formation of thrombi. Cortisone suppresses the fibrinolytic activity of the vessel walls (22) and therefore increases the tendency to thrombus formation. As a consequence, cortisone should be given only when absolutely necessary for the underlying disease.

Treatment of RDS is very unsatisfactory: the syndrome does not respond to ordinary methods of respiratory therapy, but positive end expiratory pressure appears helpful in combating atelectasis and hypoxemia. Use of extracorporeal oxygenator alone or in combination with pulmonary lavage and alveolar instillation of an artificial surfactant, as suggested by Safar et al. (25), should be tried in established RDS.

We feel that the important aspect of this study is to link RDS to TNBE, thereby shedding some new light on the pathogenesis of both conditions. Obviously definite knowledge of the etiology is still lacking, and as long as that is the case, therapy will give poor results. For the time being, therefore, prevention of suspected initiating or aggravating factors offers more hope.

REFERENCES

1. Ashbaugh, D. G., Bigelow, D. B., Petty, T. L. & Levine, B. E.: Acute respiratory distress in adults. Lancet 2: 319, 1967.
2. Barry, W. E. & Scarpelli, D.: Nonbacterial thrombotic endocarditis. Arch Intern Med 109: 151, 1962.
3. Baumgartner, H. R., Tranzer, J. P. & Studer, A.: An electron microscopic study of platelet thrombus formation in the rabbit with particular regard to 5-hydroxytryptamine release. Thromb Diath Haemorrh 18: 592, 1967.
4. Blaisdell, F. W.: Respiratory insufficiency syndrome: clinical and pathological definition. J Trauma 13: 195, 1973.
5. Blaisdell, F. W. & Schlobohm, R. M.: The respiratory distress syndrome: a review. Surgery 74: 251, 1973.
6. Borgstrom, S., Gelin, L. E. & Zederfeldt, B.: The formation of vein thrombi following tissue injury: an experiment study in rabbits. Acta Chir Scand (Suppl) 247: 1, 1959.
7. Bygdeman, S. & Wells, R.: Studies on platelet adhesiveness, blood viscosity and the microcirculation in patients with thrombotic disease. J Atheroscler Res 10: 33, 1969.
8. Carlsson, L. A., Liljedahl, S. O. & Wirsen, C.: Blood and tissue changes in the dog during and after excessive free fatty acid mobilization. Acta Med Scand 178: 81, 1965.
9. Cronberg, S.: Evaluation of platelet aggregation. Coagulation 3: 139, 1970.
10. Cronberg, S. & Nilsson, I. M.: Coagulation studies after administration of a fat emulsion, Intralipid. Thromb Diath Haemorrh 18: 664, 1967.
11. Cronberg, S., Robertsson, B., Nilsson, I. M. & Nilehn, J. E.: Suppressive effect of dextran on platelet adhesiveness. Thromb Diath Haemorrh 16: 384, 1966.
12. Fleming, W. H. & Bowen, J. C.: The use of diuretics in the treatment of the early wet lung syndrome. Ann Surg 175: 505, 1972.
13. Greenfield, L. J., Reif, M. E., Coalsson, J. J., McCurdy, J. R. & Elkins, R. C.: Comparative effects of interstitial pulmonary edema produced by venous hypertension or hemodilution in perfused lungs. Surgery 71: 857, 1972.
14. Hovig, T.: Release of a platelet aggregating substance (adenosine diphosphate) from rabbit blood platelets induced by saline "extract" of tendons. Thromb Diath Haemorrh 9: 264, 1963.
15. Hucker, H. B. & Porter, C. C.: Studies on the metabolism of amitriptyline (Abstract). Fed Proc 20: 172, 1961.
16. Isacson, S.: Effect of prednisolone on the coagulation and fibrinolytic systems. Scand J Haematol 7: 212, 1970.
17. Iversen, L. L.: Inhibition of nor adrenalin uptake by drugs. J Pharm Pharmacol 17: 62, 1965.
18. Lidbrink, P., Jonsson, G. & Fuxe, K.: The effect of imipramine-like drugs and antihistamine drugs on uptake mechanisms in the central noradrenaline and 5-hydroxytryptamine neurons. Neuropharmacology 10: 521, 1971.
19. Lundh, B., Gronkvist, U. & Olsson, A. M.: Should albumin be so widely used? Lakartidningen 73: 1319, 1976.
20. Marshall, A. & Moore, K.: Pulmonary disease after amitriptyline overdosage. Br Med J 1: 716, 1973.
21. Newton, K. W.: Physostigmine salicylate in the treat

ment of tricyclic antidepressant overdosage JAMA 231 941 1975

22 Nilsson I M Fibrinolysis thrombolysis and defibrination In Haemorrhagic and thrombotic diseases chapter 3 Wiley New York 1974

23 Orell S R Lung pathology in respiratory distress following shock in the adult Acta Pathol Microbiol Scand (A) 79 65 1971

24 Pratt P C Oxygen toxicity as a factor J Trauma 8 854 1968

25 Safar P Grenvik A & Smith J Progressive pulmonary consolidation review of cases and pathogenesis J Trauma 12 955 1972

26 Saldeen T The microembolism syndrome Forens Sci I 179 1972

27 Vijeyaratnam G S & Corrin B Fine structural alterations in the lungs of iprindole treated rats J Pathol 114 233 1974

28 Wilson I C Gambill S M & Sandifer M G Loeffler's syndrome occurring during imipramine therapy Am J Psychiatry 119 892 1963

29 Wooley C F Baba N & Ryan J M Nonbacterial thrombotic endocarditis Arch Intern Med 1[illegible] 126 1970

30 Zucker M B Effect of heparin on platelet function Thromb Diath Haemorrh 33 63 1974

Acta Med Scand 202 213–219 1977

The Adrenocortical Response to Angiotensin II Infusion in Anephric and Non-Nephrectomized Patients on Regular Hemodialysis

K Ølgaard S Madsen and M Hammer

From Medical Department P Division of Nephrology Rigshospitalet Copenhagen Denmark

ABSTRACT In the present study 8 anephric and 4 non nephrectomized patients were stimulated with angiotensin II (A II) In 5 of the anephric patients an increased plasma aldosterone concentration (PAC) in response to ACTH stimulation had previously been demonstrated After A II stimulation, all 8 anephric patients responded with a significant rise in PAC although the increase was less pronounced than in 4 non nephrectomized patients In both groups of patients the increase in PAC was correlated to the increase in diastolic and systolic BP and to the A II dose Furthermore in the non nephrectomized patients, the plasma renin activity showed a significant decline which was inversely correlated to the increase in PAC When all 12 patients regardless of the difference in remaining renin–angiotensin system were considered as one population the variable basal levels of PAC correlated significantly to the increase in PAC during A–II and ACTH stimulation It is concluded that the adrenals of anephric man respond to A II with an increase in PAC and that the reason for a lower response appears to be the lack of the renin–angiotensin system

In normal man aldosterone secretion is regulated mainly by ACTH potassium and the renin–angiotensin system (2 15 18 19 23 28 29 48 49 59) the latter being the most important regulatory mechanism (14 51 59) The anephric patient offers an excellent opportunity for examining a direct effect on the aldosterone secretion as the renal renin is eliminated (3 4 11 43 57) and the plasma renin activity (PRA) is very low and unresponsive to stimuli (11 32 56 57) Multiple investigations on such patients have been carried out but many of the results are contradictory (3 10 11 21 32 33 38 54 55 57 58 62 65 68) One would expect the anephric patient like normal man to react with an increase in the plasma aldosterone concentration (PAC) after infusion of angiotensin II (A II) However this has not been confirmed since either absent (38 58 62) or blunted responses (21 48 55) have been reported

In previous investigations we have found that anephric patients are able to increase PAC in response to ACTH and potassium stimulation (65 68 69) The present investigation was therefore undertaken to examine whether these anephric patients with previously proven reactivity of their adrenals could react with an increase in PAC during A II infusion The study was performed by infusing A II into 8 anephric patients and comparing the results with those in 4 non nephrectomized patients all on regular hemodialysis

PATIENTS

The A II infusions were carried out in 12 patients with terminal renal failure on regular hemodialysis In 8 of the patients (6 females 2 males) bilateral nephrectomy had previously been performed The mean age in the anephric group was 44 5 years (range 28–59) The mean time on regular hemodialysis was 37 6 months (range 11–84) and the mean time since bilateral nephrectomy was 28 4 months (range 4–76) The nephrological diagnoses were chronic glomerulonephritis 3 malignant nephrosclerosis 3 polycystic kidney disease 2

The 4 non nephrectomized patients (2 females 2 males) had a mean age of 51 8 years (range 40–58) Their mean time on regular hemodialysis was 40 5 months (range 17–64) and the nephrological diagnoses were chronic glomerulonephritis 3 chronic interstitial nephropathy 1

All patients were hemodialysed twice a week and received a diet containing on an average 0 8 g /kg

Table I *Mean±S E M values of 8 anephric patients during an angiotensin II infusion*

Time (min)	A II dose (ng/kg min⁻¹)	Aldosterone (pg/ml)	Diastolic BP (mmHg)	Systolic BP (mmHg)	PRA (ng/ml h⁻¹)	Potassium (mmol l)	Sodium (mmol l)	[illegible]
0		51 6 9	82 5 4 4	138 1 5 2	0 11 0 04	5 7 0 3	138 0 1 3	11 4 1 4
0		55 7 3	83 1 3 8	138 8 5 6	0 07 0 02	5 7 0 3	136 6 1 8	10 9 1 1
0	0 0	49 7 6	81 9 5 0	140 6 6 1	0 11 0 04	5 8 0 3	137 5 1 3	10 6 1 1
10	1 0	97 22 1	87 5 5 2	148 1 5 5	0 16 0 04	6 0 0 3	137 3 1 1	10 2 1 4
20	1 0	106 25 1	90 0 5 2	149 4 4 8	0 08 0 02	6 1 0 3	137 1 1 2	9 6 1 1
30	2 0	125 31 8	90 6 5 2	150 6 5 8	0 08 0 05	6 1 0 3	136 4 1 4	8 8 1 1
40	2 0	132 35 6	91 3 4 4	152 5 5 4	0 07 0 05	6 2 0 3	136 6 1.3	8 7 0 9
60	4 0	148 46 1	94 4 5 1	162.5 5 0	0 11 0 03	6 2 0 3	135 9 1 4	8 1 0 9
80	6 0	155 50 6	96 9 6 0	168 8 5 4	0 04 0 02	6 1 0 3	136 1 1 4	8 7 1 1
100*	8 0	178 59 1	95 0 6 2	170 7 5 7	0 11 0 03	6 3 0 2	135 4 1.5	6 8 1 0
120	10 0	191 63 6	97 9 6 8	175 0 6 5	0 07 0 02	6 1 0 2	135 7 1 8	7 9 0 9
140°	12 0	132 57 9	96 0 5 6	173 0 9 7	0 08 0 02	5 9 0 1	133 8 1 3	7 6 1 0

* 7 patients ° 5 patients

b wt 50 mEq sodium 50 mEq potassium and about 800 ml of fluid per day All patients had a normal BP all were in good condition and did not receive hormones or any other treatment than the dialyses vitamins and phosphate binders Informed consent was obtained from all patients

METHODS

The reactivity to angiotensin II stimulation All A II infusions were carried out at 8 a m 3 hours before the start of the hemodialysis and 3 days after the previous dialysis The A II (Hypertensin® Ciba) was dissolved in isotonic glucose at a concentration of 166 ng/ml

All patients were in the supine position throughout the study After 30 min rest 3 venous blood samples were drawn and the BP was measured at 5 min intervals as

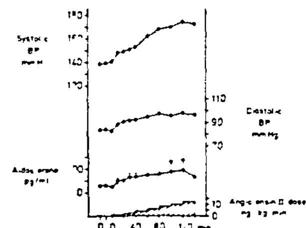


Fig 1 Plasma aldosterone concentration diastolic and systolic BP in 8 anephric patients during an angiotensin II stimulation (mean±S E M) The asterisks indicate that the infusion was terminated in one and two patients respectively due to an increase of more than 20 mmHg in diastolic BP

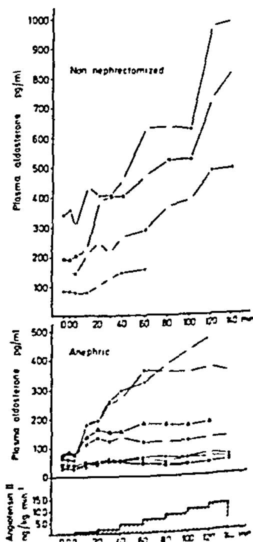


Fig 2 Individual increase in plasma aldosterone concentration in 8 anephric and 4 non-nephrectomized patients during angiotensin II stimulation

Table II *Mean±S.E.M. values of 4 non-nephrectomized uremic patients during an angiotensin II infusion*

Time (min)	A II dose (ng/kg/min)	Aldosterone (pg/ml)	Diastolic BP (mmHg)	Systolic BP (mmHg)	PRA (ng/ml/h)	Potassium (mmol/l)	Sodium (mmol/l)	Cortisol (μg/100 ml)
0		188 56.2	76.2 5.2	136.3 8.9	1.37 0.23	5.3 0.2	132.3 0.9	13.4 1.7
0		195 58.7	78.8 5.5	137.5 8.5	1.40 0.18	5.3 0.2	132.0 1.1	12.3 1.3
0	0.0	183 47.6	78.8 4.3	138.8 7.7	1.30 0.40	5.4 0.2	134.5 1.9	12.3 1.4
10	1.0	237 74.2	80.0 5.4	151.3 6.6	1.14 0.25	5.5 0.2	133.0 0.9	12.0 1.4
0	1.0	288 70.7	86.3 7.5	155.0 8.9	1.20 0.17	5.6 0.2	132.3 0.6	11.2 0.9
30	2.0	290 70.0	87.5 8.3	155.0 8.4	1.20 0.20	5.6 0.2	131.0 0.9	10.1 0.8
40	2.0	319 71.2	90.0 11.0	162.5 10.3	1.01 0.11	5.5 0.2	131.3 1.3	9.4 0.9
60	4.0	390 103.8	92.5 7.5	166.3 5.5	1.00 0.07	5.7 0.3	132.3 1.5	9.1 0.9
80	6.0	513 77.9	91.7 9.3	166.7 6.7	0.94 0.05	5.5 0.2	131.0 1.5	10.1 0.8
100	8.0	518 69.8	91.7 9.3	166.7 4.4	0.73 0.13	5.5 0.3	130.3 1.5	9.3 0.9
120*	10.0	736 141.1	93.3 8.3	168.3 4.4	0.60 0.02	5.4 0.3	129.3 0.9	9.5 1.0
140	12.0	779 147.4	98.3 8.3	186.7 1.7	0.65 0.04	5.4 0.2	130.3 0.9	9.4 0.8

* 3 patients

control values. At time zero the A II infusion was initiated in a dose of 1 ng/kg/min. The dose was increased 20 min later to 2 ng/kg/min and then with 2 ng/kg/min every 20 min until a dose of 12 ng/kg/min. BP was measured every 5 min and the infusion was terminated if the diastolic BP had risen more than 20 mmHg above the control level. Simultaneously blood samples were obtained every 10 min for the first 40 min and then at intervals of 20 min just before each subsequent stepwise increase in the rate of A II infusion. Plasma aldosterone, potassium, sodium, cortisol and renin activity were measured in all samples.

PAC was measured by a radioimmunoassay (64), PRA according to the method of Haber et al. (22), plasma cortisol by competitive protein binding technique (67), potassium and sodium by flame photometry and BP by a standard sphygmomanometer (Tycor®). Statistical significance was determined by means of Wilcoxon's test for paired data.

The reactivity to ACTH stimulation was examined in 5 of the 8 anephric and in all 4 non-nephrectomized patients with a technique previously described (68). They received synthetic ACTH (Synacthen®) in three subsequent single doses of 125.0, 62.5, 62.5 μg and the plasma concentrations of aldosterone, cortisol, sodium and potassium as well as the PRA were measured every 20 min.

The aldosterone antibody was obtained from the National Institute of Arthritis and Metabolic Diseases, Bethesda, Maryland, USA.

RESULTS

Anephric patients during angiotensin II infusion

The mean basal resting BP was 139/82 mmHg (range 120/65–165/100) (Table I). During the A II infusion the mean diastolic BP increased gradually (Fig. 1) to a maximum value of 98 mmHg ($p<0.02$) and the mean systolic BP to 175 mmHg ($p<0.001$).

Due to an increase of more than 20 mmHg in diastolic BP the A II infusion was terminated in one patient at an infusion rate of 6.0 ng/kg min (after 80 min) and in 2 patients at an infusion rate of 10 ng/kg min^{-1} (after 120 min), while the remaining

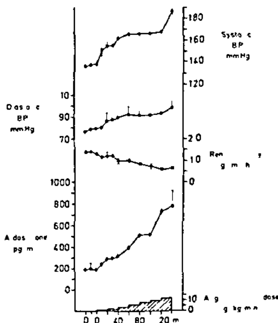


Fig. 3. Plasma aldosterone concentration, renin activity, diastolic and systolic BP in 4 non-nephrectomized uremic patients during angiotensin II stimulation (mean+S.E.M.). The asterisks indicate that the infusion was terminated in one patient due to an increase of more than 20 mmHg in diastolic BP.

Table III *Basal level (B) of and maximal increase (M) in plasma aldosterone concentration in anephric and non nephrectomized patients after stimulation with angiotensin II and ACTH*

Pat no	Aldosterone (pg ml)			
	After A II infusion		After ACTH stimulation	
	B	M	B	M
Anephric				
1	26	4	34	41
2	28	37	23	29
3	39	10	17	38
4	42	9	32	64
5	60	416		
6	60	311		
7	78	59	71	64
8	78	105		
Non nephrectomized				
9	83	75	23	43
10	141	359	309	453
11	196	649	203	720
12	335	666	52	141

5 patients continued until a dose of 12 0 ng/kg min [1] (140 min)

The mean basal PAC before the A II infusion was pg/ml (range 26–78) A significant increase 0 001) to a mean maximum value of 191 pg/ml was seen during the infusion As shown in Fig 2 the increase in PAC varied between patients within a range of 9–416 pg ml The increase in mean PAC was correlated to the dose of A II as well as to the rise in diastolic and systolic BP ($p<0$ 001)

During the whole investigation PRA was very low and remained unchanged The plasma cortisol concentration showed a slight non significant decrease ($p>0$ 05) during the A II infusion No changes were seen in the plasma potassium or sodium concentrations (Table I)

Non nephrectomized patients during angiotensin II infusion

The mean basal resting BP was 138/78 mmHg (range 120/70–160/95) (Table II) In one of the 4 patients the infusion was terminated after 60 min (at an A II dose of 4 0 ng/kg min) due to an increase in diastolic BP of 30 mmHg In the whole group the mean diastolic BP rose to 98 mmHg (p 0 01) (Fig 3) and the mean systolic BP to 187 mmHg ($p<0$ 001)

The mean basal PAC before A II infusion was 189 pg/ml (range 83 335) (Table II) During A II infusion the mean PAC increased to a maximum of 779 pg/ml ($p<0$ 001) The rise in PAC between individuals ranged from 75 to 666 pg ml (Fig) Significant correlations were found between the increase in PAC the dose of A II the diastolic and the systolic BP ($p<0$ 001)

The mean PRA declined slowly during the A II infusion ($p<0$ 05) and was inversely correlated to the dose of A II and to the increase in PAC ($p<0$ 001)

As in the anephric group a slight insignificant decline in plasma cortisol was noted (Table II) Plasma potassium and sodium remained unchanged

Correlation between the PAC response to A II and ACTH stimulation

ACTH stimulation was performed in 5 of the 8 anephric patients (Table III) The mean maximal increase in PAC during the ACTH stimulation was 47 2 pg/ml (range 29–64) In all anephric patients a significant correlation ($p<0$ 05) was found between the basal levels of PAC and the maximal increase in PAC after A II infusion as well as after ACTH stimulation

In the 4 non nephrectomized patients the maximal increase in PAC after ACTH was 329 2 pg ml (range 43–720) and as in the anephric group

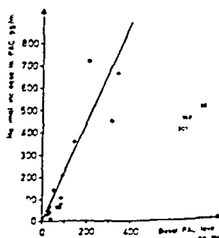


Fig 4 Correlation between basal levels of plasma aldosterone and the maximal increase in plasma aldosterone during angiotensin II stimulation (●) and ACTH stimulation (○) in 8 anephric and 4 non nephrectomized patients on regular hemodialysis

significant correlation ($p<0.001$) was found between the basal levels of PAC and the maximal increase in PAC during the A II infusion and the ACTH stimulation

Furthermore the relation between basal levels of PAC and the maximal increase in PAC during A II infusion and ACTH stimulation respectively was identical in anephric and non nephrectomized patients regardless of the differences in basal PAC (Fig 4) and remaining renin–angiotensin system This may indicate that the two groups of patients could be considered as one population

DISCUSSION

The consensus of opinion is that A II has a direct action on the adrenal cortex (1 7 25 35 44 45 50 52 63) over and above its vascular effect (9 12 16 24 26 61) Despite this many experimental and clinical situations are reported to show a discrepancy between the values of the renin–angiotensin system and PAC (6 8 31 40 46 47 49) Beside the renin–angiotensin system it is found that potassium ACTH extracellular volume sodium (17, 20 30 53 60) and maybe unidentified factors (6 14 30 31 34 40 46 47) participate in the regulation of aldosterone It has also been shown that the aldosterone response to an A II infusion may depend on the sodium balance (1 15 44 59 61) the potassium balance (23 24 59) and the ACTH levels (5 42) The aldosterone response to an ACTH stimulation may likewise depend on the same factors (2 60 68)

Many investigations like the present have found a low PAC level in anephric patients (21 32 54–57 58 66) although PAC within normal range also has been described (36 37 39 41) Despite the low PAC level anephric patients react with an increase in PAC following an increase in plasma potassium (3 11 32 55–57 69) in intracellular potassium (65) and in ACTH stimulation (38 41 68) although the latter response has not been found by all (21 58) Considering that anephric patients are able to increase the PAC following various stimuli it is surprising that the response to an A II infusion is generally described as absent or blunted (21 38 48 55 58 62) especially in view of the strong stimulatory effect of this hormone in normal man

In the present investigation the effect of an A II infusion was studied mainly in anephric patients who previously had demonstrated an increase in PAC following ACTH stimulation At variance with other investigations using the same A II dose (21 38 48 55 58 62) a clear increase in PAC was found in the present study corresponding to the increase previously shown after ACTH stimulation The increase in PAC correlated with the dose of A II and the increase in BP The PCC showed a slight fall in accordance with the normal circadian rhythm (68) and plasma potassium and sodium were unchanged during the study Consequently neither of the other three known stimulating factors could explain the increase in PAC

In non nephrectomized patients with considerably higher PAC and PRA levels a much more pronounced increase in PAC was seen during the A II infusion in accordance with other investigations (32 62) Simultaneously there was a fall in PRA as demonstrated in normal subjects during A II infusion (27) The PCC potassium and sodium followed the same pattern as in anephric patients

When all dialysis patients with and without preserved renal renin–angiotensin system were considered as a single population their responses in PAC to A II and ACTH stimulation respectively correlated significantly ($p<0.001$) to their basal levels of PAC Based on this finding it is suggested that the reactivity of the adrenals depends upon stimulating factors that are already present This is in accordance with investigations in normal man (30) demonstrating that the ability of the adrenals to react with an increase in PAC depends not on just one but on multiple factors among which a preserved renal renin–angiotensin system is apparently of major importance

We therefore conclude that the adrenals of anephric patients are able to respond with an increase in PAC to various types of stimulation including A II The reason for the lower PAC response in anephric patients may be the low prestimulatory level of PAC probably caused by lack of the renin–angiotensin system

ACKNOWLEDGEMENTS

The study was supported by grants from the Danish Medical Research Council King Christian X's Foundation and the Einar Willumsen Memorial Foundation

REFERENCES

1 Aguilera G White A & Marusic E T docrinol (Kbh) 80 104 1975

Table III *Basal level (B) of and maximal increase (M) in plasma aldosterone concentration in anephric and non nephrectomized patients after stimulation with angiotensin II and ACTH*

Pat no	Aldosterone (pg/ml) After A II infusion B	M	After ACTH stimulation B	M
Anephric				
1	26	47	34	41
2	28	37	23	29
3	39	10	17	38
4	42	9	32	64
5	60	416		
6	60	333		
7	78	59	71	64
8	78	105		
Non nephrectomized				
9	83	75	23	43
10	141	359	309	453
11	196	649	203	720
12	335	666	52	141

5 patients continued until a dose of 12 0 ng/kg min⁻¹ (140 min)

The mean basal PAC before the A II infusion was 1 pg/ml (range 26–78) A significant increase -0 001) to a mean maximum value of 191 pg/ml was seen during the infusion As shown in Fig 2 the increase in PAC varied between patients within a range of 9–416 pg/ml The increase in mean PAC was correlated to the dose of A II as well as to the rise in diastolic and systolic BP ($p<0.001$)

During the whole investigation PRA was very low and remained unchanged The plasma cortisol concentration showed a slight non significant decrease ($p>0.05$) during the A II infusion No changes were seen in the plasma potassium or sodium concentrations (Table I)

Non nephrectomized patients during angiotensin II infusion

The mean basal resting BP was 138/78 mmHg (range 120/70–160/95) (Table II) In one of the 4 patients the infusion was terminated after 60 min (at an A II dose of 4 0 ng/kg min⁻¹) due to an increase in diastolic BP of 30 mmHg In the whole group the mean diastolic BP rose to 98 mmHg (p 0 01) (Fig 3) and the mean systolic BP to 187 mmHg ($p<0.001$)

The mean basal PAC before A II infusion was 189 pg/ml (range 83–335) (Table III) During the A II infusion the mean PAC increased to a maximum of 779 pg/ml ($p<0.001$) The rise in PAC between individuals ranged from 75 to 666 pg/ml (Fig *Significant correlations were found between the increase* in PAC the dose of A II the diastolic and the systolic BP ($p<0.001$)

The mean PRA declined slowly during the A II infusion ($p<0.05$) and was inversely correlated to the dose of A II and to the increase in PAC ($p<0.001$)

As in the anephric group a slight insignificant decline in plasma cortisol was noted (Table II) Plasma potassium and sodium remained unchanged

Correlation between the PAC response to A II and ACTH stimulation

ACTH stimulation was performed in 5 of the 8 anephric patients (Table III) The mean maximal increase in PAC during the ACTH stimulation was 47 2 pg/ml (range 29–64) In all anephric patients a significant correlation ($p<0.05$) was found between the basal levels of PAC and the maximal increase in PAC after A II infusion as well as after ACTH stimulation

In the 4 non nephrectomized patients the maximal increase in PAC after ACTH was 339 2 pg/ml (range 43–720) and as in the anephric group a

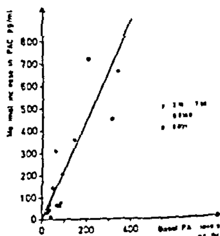


Fig 4 Correlation between individual basal levels of plasma aldosterone and the maximal increase in plasma aldosterone during angiotensin II infusion (B and ACTH stimulation (C) in 8 anephric and 4 non nephrectomized patients on regular hemodialysis

57 Weidmann P Maxwell M H & Lupu A N Ann Intern Med 78 13 1973
58 Williams G H Bailey G L Hampers C L Lauler D P Merrill J P Underwood R H Blair West J R Coghlan J P Denton D A Scoggins B A & Wright R D Kidney Int 4 280 1973
59 Williams G & Dluhy R G Am J Med 53 595 1972
60 Williams G H Dluhy R G & Underwood R H Clin Sci 39 489 1970
61 Williams G H Hollenberg N K & Braley L M Endocrinology 98 1343 1976
62 Woods T J C McCaa R E Bower J D & McCaa C S Trans Am Soc Artif Intern Organs 20 154 1974
63 Yamamoto Y Jpn Heart J 16 404 1975
64 Ølgaard K Scand J Clin Lab Invest 35 31 1975
65 — Acta Med Scand 198 213 1975
66 Ølgaard K & Ladefoged J In Radionuclides in nephrology (ed K Winkel M D Blaufox and J L Funck Brentano) 3 159 Thieme Verlag Stuttgart 1974
67 Ølgaard K & Madsen S Clin Biochem 9 265 1976
68 — Acta Med Scand 201 77 1977
69 — Acta Med Scand In press 1977

Acta Med Scand 202 221–224 1977

The Hepatic Conversion of Vitamin D in Alcoholics with Varying Degrees of Liver Affection

B Lund O H Sorensen M Hilden and B Lund

From Department of Medicine E Frederiksberg Hospital and the Department of Medicine Hvidovre Hospital Copenhagen the Department of Medicine Maribo and the Department of Orthopaedic Surgery Frederiksborg County Hospital Hillerød Denmark

BSTRACT The seasonal variations in circulating 5-hydroxycholecalciferol (25 HCC) were studied in 02 alcoholics with fatty liver disease without istologic signs of cirrhosis and in 35 patients with coholic cirrhosis The mean levels were compared ith those of normal persons Alcoholics had gener lly lower 25-HCC values than the controls particu rly in the summer This was primarily explained by isufficient diet and reduced exposure to sunshine he ability of the liver to hydroxylate in the 25-osition was studied in three groups of alcoholics with) fatty liver disease without cirrhosis 2) com ensated cirrhosis 3) severely incompensated liver irrhosis All three groups exhibited a significant in rease in serum 25 HCC following the peroral dministration of cholecalciferol at a dose of 1200 U aily for 7 days Similar rises were seen 7 days after a ingle injection of 10000 U cholecalciferol This indi ates a normal intestinal absorption of vitamin D ven in advanced alcoholic liver disease and is incon istent with a severely damaged 25 hydroxylation apacity in these patients Osteomalacia due to im aired liver hydroxylation of vitamin D can hardly xplain the increased fracture rate and the decreased one mass which have been described in alcoholics

After absorption from the intestine or derivation by ltraviolet light irradiation from the skin precursor holecalciferol is hydroxylated in the liver to 25 ydroxycholecalciferol (25 HCC) the major cir ulating form of the vitamin (14) This metabolite s further hydroxylated in the kidney to the most otent form of vitamin D known 1 25-dihydroxy holecalciferol (5) The liver thus has a central posi ion in the metabolism of vitamin D which might xplain some of the bone abnormalities that have been reported in patients with chronic liver dis eases So far most interest has concentrated on patients with primary biliary cirrhosis a rather rare disease These patients often develop osteoporosis and osteomalacia due to malabsorption of calcium and vitamin D (1 9 18 21) or a defect in the hepatic metabolism of the vitamin (20) Liver affec tion in chronic alcoholism is on the other hand a much more common disease but here the state of the skeleton and the metabolism of vitamin D have been less intensively studied The results are furth ermore controversial Some studies show a de creased bone mass in alcoholics (3 4 13 16) while others point at a normal skeleton in these patients (15) Measurements of 25 HCC in alcoholics with out cirrhosis have demonstrated normal (8) or low values (10) In one study (19) serum 25 HCC was low in female alcoholics without cirrhosis but not in males There seems to be general agreement however that 25 HCC is low when alcoholic cirrhosis has developed (8 10 11)

The present study was undertaken to measure circulating 25 HCC in chronic alcoholics with 1) fatty liver disease without cirrhosis 2) com pensated cirrhosis 3) cirrhosis with severe liver failure The ability of the liver to hydroxylate in the 25 position was studied in these patients Cholecal ciferol was administered both by the oral and by the intramuscular route to exclude differences in in testinal absorption

PATIENTS AND METHODS

Circulating 25 HCC was measured in 102 patients with varying degrees of fatty liver disease but without any

2 Avioli L U & Haddad J G Metabolism 22 507 1973
3 Collesson L Grilhat J P Mathieu J & Laurent J Presse Méd 45 2571 1965
4 Dalen N & Lamke B Acta Orthop Scand 47 469 1976
5 Fraser D R & Kodicek E Nature 228 764 1970
6 Haddad J G & Chyu K J J Clin Endocrinol Metab 33 992 1971
7 Haddad J G & Stamp T B C Am J Med 57 57 1974
8 Hepner G W Roginsky M & Moo H F In Vitamin D and problems related to uremic bone disease (ed A W Norman K Schaefer H G Grigoleit D von Herrath and E Ritz) p 325 W de Gruyter Berlin 1975
9 Kehayoglou A K Holdsworth D D Agnew J F Whelton M J & Sherlock S Lancet 1 715 1968
10 Long R G Skinner R K Meinhard E Wills M R & Sherlock S In XIth Meeting of the European Association for the Study of the Liver p 478 Proceedings Copenhagen 1976
11 Long R G Skinner R K Wills M R & Sherlock S Lancet 2 650 1976
12 Lund B Sorensen O H & Christensen A B Lancet 2 300 1975
13 Nilsson B L Acta Chir Scand 136 383 1970
14 Ponchon G Kennan A L & DeLuca H F J Clin Invest 48 2032 1969
15 Roginsky M S Zanzi I & Cohn S H In Calcified tissues 1975 (ed S P Nielsen and E Hjørting Hansen) p 386 FADL Copenhagen 1976
16 Saville P D J Bone Joint Surg (Am) 47 492 1965
17 Scott K G Smyth F S Peng C T Rosa W A Stevenson I A & Castle J N Strahlentherapie (Suppl) 60 317 1965
18 Thompson G R Lewis B & Booth C C J Clin Invest 45 94 1966
19 Verbanck M Brauman J Verbanck J A Mertens J P In XII European Symposium on Calcified Tissues York 1976 In press 1977
20 Wagonfeld J B Nemchausky B A Bolt M Horst J V Boyer J L & Rosenberg I H Lancet 2 391 1976
21 Whelton M J Kehayoglou A K Agnew J E Turnberg L A & Sherlock S Gut 12 978 1971

Acta Med Scand 202 225–229 1977

Clinically Undiagnosed Active Tuberculosis

Experience from an Autopsy Material

Asger Juul

From the Department of Pathology Central Hospital Esbjerg Denmark

ABSTRACT The incidence of clinically undiagnosed active tuberculosis (CUDAT) was 0 1% in an autopsy material comprising about 75% of all autopsies carried out in Denmark during the period 1 1 1969–1 1 1974 This incidence, corresponding to one case in every 895 autopsies is a minimum value From the present study it is apparent that the miliary form is predominant CUDAT is most often found in elderly patients from medical units The most common cause of death is the active tuberculosis, especially the miliary form Fever often occurs during the stay in hospital and is an almost invariable sign in miliary tuberculosis Many of the patients had been treated with corticosteroids and/or cytostatics for associated diseases especially malignancies

The occurrence of clinically undiagnosed active tuberculosis (CUDAT) in autopsy materials has been the subject of many investigations (3 5 7 8 9 10 14 15 16 17 18 19 20 21 23 24 26) which have consistently shown that surprisingly often a diagnosis of active tuberculosis is not made until post mortem

In Denmark these problems have most recently been elucidated by Helms et al (10) who were concerned with the period 1960–62

Since that time there has been an appreciable decrease in the morbidity and mortality of tuberculosis in Denmark (25) It therefore seemed of interest to make another study of the incidence of CUDAT in an autopsy material and to ascertain to what extent under which circumstances and in which forms CUDAT can be expected to be found at autopsy to-day

MATERIAL AND METHODS

Active tuberculosis in this paper is taken to mean epitheloid cell granulomatous lesions in which typical tubercle bacilli are demonstrated by Ziehl Neelsen staining by fluorescence microscopy or by culture Cases with no histological material at disposal but in which cultures were positive were also interpreted as active tuberculosis

CUDAT is taken to mean cases in which tuberculosis has not been listed as a diagnosis in the clinical record On the other hand the study did not include patients in whom there was such strong suspicion of active tuberculosis that antituberculous treatment had been instituted

The material comprises all autopsies except the medicolegal ones carried out in those Danish departments of pathology where autopsies have been performed throughout the period 1 1 1969–1 1 1974 a total of 20 departments in all parts of Denmark During that period a total of 76978 autopsies were carried out in these departments

With the aid of diagnostic files and/or continuous reviews of autopsy records I sorted off records giving a diagnosis of tuberculosis As far as possible the method was continuous reviewing as the sources of uncertainty involved in the use of diagnostic files were realized from the outset On the basis of the autopsy records thereafter patients were excluded if their clinical records clearly showed that they had had active tuberculosis Patients were also excluded if it was apparent that no tissue had been obtained for microscopic examination or culture So were those in whom microscopic study had definitely revealed old inactive lesions After reviews of the remaining clinical records patients were excluded if it was apparent that they had had clinically diagnosed active tuberculosis (cf the definition)

Of those that now remained 18 had to be excluded as it was not possible to procure the tissue originally removed From the remaining specimens new histological sections were prepared (Ziehl Neelsen van Gieson-Hansen haematoxylin eosin staining as well as fluorescence staining (6) in cases where the Ziehl Neelsen staining was negative) In 5 cases with no histological material available there had been positive findings on culture

On the basis of the macroscopic description in the autopsy records and the revised histological examination the tuberculous lesions were classified as listed under Results

In the clinical records I also noted the age distribution

Table I Fever during stay in hospital in relation to pathological group

Pathological group	Fever during % of stay in hospital 0	1 25	26–50	51 75	76–100	Unknown	Total
A	7	0	2	2	2	3	16
B	4	0	3	6	8	1	21
C	1	1	1	2	2	0	7
D	1	0	3	0	33	1	38
E	0	0	0	1	2	1	4
Total	13	1	9	11	46	6	86

sex ratio symptoms and signs medication, presence of associated diseases causes of death and distribution on clinical units.

RESULTS

The search yielded a total of 86 cases of CUDAT which is 0 1% or 1 case in 895 autopsies In 59 cases tubercle bacilli were demonstrated by Ziehl Neelsen staining, in 11 by fluorescence staining and in 16 by culture

Pathological classification

A. Fibrocaseous tuberculosis Major confluent caseous necroses surrounded by connective tissue 16 cases (18 6%) B Fibrocaseous tuberculosis with miliary spread. Major confluent necroses of [illegible] nature surrounded by connective tissue [illegible]d several smaller tuberculous foci made up of one or more tubercles 21 cases (24 4%) C Caseous pneumonia. Bronchial spread with caseous degeneration of major areas of pulmonary tissue 7 cases (8 1%) D Miliary tuberculosis Several foci made up of one or a very few tubercles 38 cases (44.2%) E Isolated one-organ tuberculosis Extrapulmonary isolated tuberculosis 4 cases (4 7%)

Age distribution and sex ratio

Fig. 1 gives the age distribution and sex ratio of the cases With the ordinary confidence limits the distribution corresponds to that in ordinary autopsy series (11) The mean age was 70 7 years

Symptoms and signs

Owing to the retrospective nature of the study it was possible only with reasonable certainty to investigate whether the patients had had fever defined as a morning and evening temperature of 37.5°C or more As seen from Table I more than 90% of the cases with miliary tuberculosis had been running a temperature almost throughout their stay in hospital

Medication

A total of 23 patients (26 7%) had been on corticosteroids and/or cytostatics up to or during their stay in hospital (Table II) Fifteen of these 23 patients had had miliary tuberculosis

Co-existing diseases

The relation to the associated diseases listed in Table III was investigated Forty-seven patients had had co-existing diseases in most cases malignant neoplastic diseases half of which were haematological (Table IV) All patients with malignant haematological diseases had the miliary form of tuberculosis (Table IV)

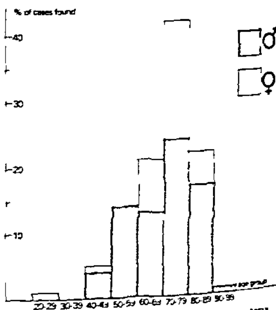


Fig 1 Age and sex distribution of the autopsy material

Tab e II *Patholog cal class f cat on of 23 pat ents t ea ed) cort costero ds and/or cytostat cs*

	Pa holog cal group				
	A	B	C	D	Total
Cort co tero ds	?	2	3	14	21
Cyto ta cs	1	2	1	5	9
Total no of pats trea ed	?	3	3	15	23

Causes of death

Table V gives the d stribut on on the presumed causes of death Tuberculosis was the most com mon cause of death being the primary cause in 46 pat ents (53 5%) Of the 46 cases mil ary tubercu los s was the primary cause of death in 27 (31 4%) fibrocaseous tuberculosis with m l ary spread in 12 caseous pneumon a in 5 and isolated one organ tuberculos s in 2 cases

A cord ng to the Dan sh Tuberculosis Index about 300 pat ents in Denmark d ed of act ve tuber cu os s during the period 1 1 1969–1 1 1974 Thus n about 15% of the pat ents who d ed of act ve tuberculos s during this period the prima. cause of dea h was und agnosed act ve tuberculos

D s b t on on cl n al i n ts

Seventy four pat ents (86%) had been in med cal units 6% in surgical units and 7 (8%) in rad um ce es or chron c care un ts

DISCUSSION

The f nd ng of CUDAT in 86 out of 76 978 autops es is low compared with other stud es (3 5 7 8 9 10 14 15 16 17 18 19 20 21 23 24 26)

In a Dan sh prospective study of 1 033 autops es during the period 1960 through 1962 Helms et al (10 found 12 cases of CUDAT correspond ng to an ncidence of 1 2% The causes of the present low inc dence m ght be a less su ted and/or insuff cient autopsy technique a def c ent method of registra t on or an actually lo ver morb d ty of tuberculos s during the study period (25) On the other hand it is less l kely that cases of CUDAT escape autopsy to any major extent as t is a less common f nd ng in med colegal autopsies (13)

It is often d ff cult to make a d agnosis of ac t ve tuberculos s on the bas s of an ord nary macroscop c examinat on and frequently active tuberculos s is m staken at autopsy for other more commonplace inflammatory les ons neoplastic d s eases and apparently inact ve tuberculosis Conse quently there s a tendency not to obtain relevant t ssue for m croscop c study let alone culture It is advisable therefore to include culture from tuber cle bacill in the rout ne stud es in an autopsy to a much greater extent than seems to be done at pres ent Demonstrat on of active tuberculos s at au topsy would be of importance partly n tracing sources of infect on partly n examining fello v pa t ents and staff Thereby t might contribute to a further decrease in the inc dence of the d sease

Table III *Co ex st ng d seases*

	Males	Females	Total
Malignant diseases	11	7	18
Diabe es mell tus	5	4	9
Hepatic c rrhos s	5	3	8
Chron c renal d seases	6	3	9
Collagenoses	2	1	3
Total	9	18	47

Table IV *Patholog cal gro ps of t berci losis in pat ents with neoplast c d seases*

	Pathological group		
	B	C	D
Malignant haematological d seases	0	0	9
Mal gnant pulmonary tumours	?	1	0
Gastro ntest nal tumours	?	1	0
Gyn /CNS tumours	1	1	?
Total	5	3	11

Table V *Ca ses of death among 86 cases of cl n call, i nd agnosed act ve tuberci los s*

	Males	Females	Total	% of series
Tuberculos s	29	17	46	53 5
Card ovascular d seases	6	0	6	7 0
Resp ratory tra t d seases	9	3	12	14 0
Mal gnant neo-plast c d seases	11	7	18	1 0
M scellaneous	3	1	4	

Reviewing the many autopsy records it was learnt that not infrequently the diagnosis of active tuberculosis had been based merely upon the gross findings This group had to be left out of the present study although presumably it includes cases of active tuberculosis As it was necessary in some measure to base the findings upon diagnostic files despite the assumption of their varying validity it is beyond doubt that the incidence of CUDAT found in the present study is definitely a minimum value

Pathology

Miliary tuberculosis constituted 44 2% of the cases in agreement with previous studies (3 7 12 15 19 20) The explanation may be that autopsy series usually comprise elderly patients in whom the miliary type of tuberculosis is predominant (11) It may be also that miliary tuberculosis is often difficult to diagnose (20) and that not infrequently it is present in a cryptic non reactive form in elderly people (1 2 4 22 27 28)

Age distribution and sex ratio

In the present series of CUDAT the age distribution and sex ratio correspond to those found previously in an ordinary autopsy material (12) and agree with the findings of others

Symptoms and signs

According to the present findings fever is often a sign of the degree of tuberculous infection In the event of fever of unknown cause therefore the possibility of active tuberculosis should be borne in mind (4) as fever seems to be an almost invariable sign of clinically undiagnosed active miliary tuberculosis

Medication

It has previously been pointed out (4) that treatment with corticosteroids and/or cytostatics may reactivate old tuberculous foci and render the patients less resistant to exogenous infections The present study confirmed that during treatment with corticosteroids and/or cytostatics the possibility of active tuberculosis should be borne in mind

However when considering the large number of patients on steroid medication the occurrence of CUDAT does not seem to be high in this connection

Co-existing diseases

From the present investigation it is apparent that in more than 50% of the cases other co-existing diseases may have weakened the patient s general condition and thereby contributed to the eruption of the active tuberculosis This agrees with previous publications (2 9 14 21 33) which have emphasized in particular the common occurrence of malignant neoplastic diseases As mentioned in the section on medication malignant neoplastic diseases are often present simultaneously with steroid and/or cytostatic medication In these cases it is not possible to decide which has been the more important cause of the eruption of active tuberculosis but both factors presumably play an important role Diagnosing miliary tuberculosis seems to be particularly difficult in the presence of malignant haematological diseases

Causes of death

CUDAT was the most common cause of death in the present series as it has been in others (20 21) As also demonstrated previously (3 15) it is usually the miliary form of tuberculosis which is the primary cause of death

Distribution on clinical units

CUDAT is most often demonstrated in autopsies on patients from medical units (15 17 18) This may be because autopsy materials are largely derived from medical units (11) but also because the symptoms and signs of tuberculosis most often lead to admission to these very units Owing to the numerous patients on corticosteroid and/or cytostatic medication the cases of CUDAT may also be expected to be found primarily among autopsies from medical units

REFERENCES

1 Ashba J K & Boyce J M Undiagnosed tuberculosis in a general hospital Chest 61 447 1972
2 Beermann B Disseminated tuberculosis in elderly patients Acta Med Scand 190 45 1971
3 Boström K Aktiv tuberkulos i ett norrlandskt obduktionsmaterial Lakartidningen 60 2717 1963
4 Böttiger L E et al Miliartuberkulos—en bortglömd diagnos Nord Med 50 1719 1961
5 Casey T P Undiagnosed fatal tuberculosis NZ Med J 67 492 1968
6 Christoffersen P Fluoroscensmikroskopisk påvisning af tuberkelbaciller i vævssnit Ugeskr Laeger 1 69 1965

7 Fausa O Aktiv tuberkulos i ett obduktionsmaterial Tidsskr Nor Laegeforen 87 970 1967
8 Finch R G & Cope J R Unsuspected tuberculosis in general hospitals Lancet 1 1496 1973
9 Grosse H & Link M Uber klinisch latent erst durch Obduktion erkannte offenen Lungentuberkulosen Z Aerztl Fortbild 61 245 1967
10 Helms T et al Tuberkulose i et sektionsmateriale Ugeskr Laeger 50 1673 1967
11 Jaques J & Sloan J M The changing pattern of miliary tuberculosis Thorax 25 237 1970
12 Juul A Ikke erkendt aktiv tuberkulose i en retsligt autopsimateriale Ugeskr Laeger 16 940 1977
13 Juul A & Andersen I A A Rutineautopsier Ugeskr Laeger 6 323 1977
14 Kogel V & Hedinger C Uber die Haufigkeit klinish unerkannter Miliartuberkulosen im Sektionsgut Schweiz Med Wochenschr 22 793 1973
15 Lagerstrom L & Lindberg J Aktiv tuberkulos i ett tvååangt obduktionsmaterial Opusc Med 4 158 1968
16 Larsson J & Linell F Tuberculosis and mortality in Sweden Acta Tuberc Scand 34 231 1960
17 Linder L Dodsfall i kliniskt odiagnosticerad tuberkulos Lakartidningen 58 1510 1961
18 Linell F & Östberg G Tuberculosis in an autopsy material Scand J Resp Dis 47 200 1966
19 — Tuberkulosen i ett stort obduktionsmaterial Svenska Nat Foren Kvartalsskr 1 1966
20 Moberg A & Orell S R Aktiv tuberkulos i ett obduktionsmaterial Opusc Med 5 335 1960
21 Makela V et al Tuberculosis—diagnosed and undiagnosed—as a cause of death Autopsy observations Scand J Resp Dis 52 13 1971
22 Proudfoot A T et al Miliary tuberculosis in adults Br Med J 1 273 1969
23 Roberts F J et al Undiagnosed tuberculosis at autopsy Can J Public Health 62 497 1971
24 Strandgård E Tuberkulose diagnosticeret postmortelt Ugeskr Laeger 2 143 1968
25 Sundhedsstyrelsen Betænkning vedrørende kollektive tuberkuloseundersøgelser No VI 19 261 1974
26 Willgeroth C & Heissmeyer V Die Tuberkulose in einem grossen Obduktionsgut der Jahre 1962 bis 1971 Inaktive und aktive Lungentuberkulosen und Miliartuberkulosen Zentralbl Allg Path 118 37 1974
27 Zachau Christiansen B Miliærtuberkulose optrædende som malign neoplastisk sygdom Ugeskr Laeger 45 1721 1959
28 Zettergren L Kliniskt odiagnostiserad fall av reaktiv generaliserad tuberkulos Lakartidningen 58 1517 1961

LETTER TO THE EDITOR

ECHOCARDIOGRAPHY IN ACUTE AORTIC ENDOCARDITIS

Dear Sir

We wish to comment on the article in your issue 200 no 5 1976 by Alstrup and Froysaker entitled Immediate and long term results of emergency valve replacement in acute bacterial endocarditis (1)

Alstrup and Fröysaker reported 18 operated cases with acute bacterial endocarditis in the aortic valve and found 3 early deaths in the immediate postoperative phase There were also 2 deaths 2 and 18 months after operation One patient was reoperated on 6 years later because of mitral insufficiency The article does not give the number of patients diagnosed as having an acute aortic endocarditis and not operated on We completely agree with the authors that it is important to evaluate if more valves are involved preoperatively However they find it reasonable to use both catheterization and angiographic investigations to get this information and in 14 of their cases such invasive measurements were made

In a non invasive investigation and especially in an echocardiographic examination there are several typical findings in acute aortic insufficiency This has recently been reported elsewhere (2 3) We have had 8 patients with this serious disease who have been evaluated preperatively only by this non invasive technique In 2 patients aortic cusp vegetations were seen on echocardiography and in the other 6 patients also a hole was diagnosed in at least one cusp One patient also had engagement of the anterior mitral leaflet Operation was performed 2–6 months after the start of infectious symptoms and within 2 months after diagnosis of aortic insufficiency At operation the echocardiographic findings were confirmed in all cases The first patient had had a ventricular fibrillation just before the planned operation and was acutely operated on She died in the immediate postoperative course One patient has been operated on just recently The other 6 patients are all in good condition 1–5 years after operation

We find it important to evaluate patients with acute bacterial endocarditis with aortic valve engagement to see if more than one valve is involved In this evaluation we find the non invasive technique with echocardiographic investigation to be superior to the invasive examination. As these patients have an acute endocarditis an operation should be performed at the optimal time when the patient has been treated but before the heart failure has gone too far The non invasive technique makes it possible to perform repeated investigations to find this optimal time without the risks connected with a left heart catheterization in acute endocarditis

Karl Swedberg Division of Cardiology Department of Medicine I Sahlgren s Hospital Göteborg Sweden

Ingemar Wallentin Department of Clinical Physiology Sahlgren s Hospital Goteborg Sweden

REFERENCES

1 Alstrup P & Froysaker T Immediate and long term results of emergency aortic valve replacement in acute bacterial endocarditis Acta Med Scand 200 37? 1976
2 DeMaria A N et al Echography and phonography of acute aortic regurgitation in bacterial endocarditis Ann Intern Med 82 329 1975
3 Roy P et al Spectrum of echocardiographic findings in bacterial endocarditis Circulation 53 474 1976

Acta Med Scand 202 231–236 1977

Pheochromocytoma and Renovascular Hypertension

A Case Report and a Review of the Literature

A Alvestrand J Bergstrom and B Wehle

From the Department of Medicine Huddinge University Hospital Huddinge and the Department of Nephrology St Erik s Hospital Stockholm Sweden

ABSTRACT A case of hypertension with simultaneous occurrence of a para aortal pheochromocytoma and a functionally significant membranous renal artery stenosis is reported The pheochromocytoma was excised surgically and a vein patch angioplasty was performed Postoperatively the BP returned to normal Three years after surgery the patient is normotensive and urinary catecholamines are normal On the basis of this case and 27 previously reported cases of pheochromocytoma and renal artery stenosis the possible relationship between the two conditions is discussed

Some of the conditions leading to hypertension that may be surgically curable are renal artery stenosis unilateral kidney disease coarctation of the aorta pheochromocytoma Cushing s syndrome and primary aldosteronism Together these lesions do not comprise more than about 5% (8 37) but considering the very high incidence of hypertension they are not uncommon However cases with simultaneous occurrence of two potentially surgically curable causes of hypertension are rare In the literature we have found 27 cases with co existing pheochromocytoma and renal artery stenosis (2 5 7 10 11 13 14 16 18 19 20 22 23 24 26 27 29 30 31 33 35 40 41 42) All except two of them (7 27) have been successfully treated with surgery

In this paper we give an account of a patient with a pheochromocytoma and a membranous renal artery stenosis On the basis of this report and the previously reported cases of renal artery stenosis and pheochromocytoma which are briefly reviewed we discuss the possible relationship between the two conditions and the hemodynamic disorders caused by them

CASE REPORT

The patient a 24-year-old woman was admitted to the Renal Clinic St Erik s Hospital Stockholm in Oct 1970 for evaluation of renal artery stenosis In Nov 1968 her BP was recorded to 140/85 mmHg In Aug 1969 during the last trimester of her first pregnancy hypertension was discovered with a BP of 180/120 mmHg The patient had then had several attacks of severe headache and nausea She was put on hydralazine 25 mg three times daily and was thereafter asymptomatic The pregnancy was in other respects normal and delivery was uncomplicated

When the postpartum BP did not return to normal the patient was referred to her local hospital for investigation On admission her BP was 150/100 mmHg Apart from the endogenous creatinine clearance 47 ml/min/1 73 m² BSA the routine laboratory findings were normal as were urinary catecholamines and i v pyelography The hypertension was considered essential and the patient was discharged without therapy

Because of complaints of headache and nausea the patient was readmitted in June 1970 BP was then 160–190/110–120 mmHg and treatment with propranolol 40 mg four times daily was started A retrograde femoral aortography was performed in Sept 1970 The left kidney and renal artery were normal The right kidney was markedly smaller than the left The right renal artery was divided into two branches at about 2 cm distal from its junction with the aorta A stenosis with a diameter of approximately 1 mm was seen in the superior branch 2 cm distal from the division A slight narrowing was found also in the lower branch

Because of these findings the patient was referred to the Renal Clinic on Oct 10 1970 She was in good general condition gave a history of progressively increasing fatigue and attacks of palpitations but had no other complaints

Physical examination revealed a pulse rate of 95/min Supine BP was 190/120 mmHg and the standing BP 170/110 No signs of heart failure or murmurs or other abnormalities were noted Hb was 12 7 g/100 ml WBC 9 700/mm³ and ESR 18 mm/h Serum creatinine was 1 1 mg/100 ml BUN 15 mg/100 ml Serum electrolytes were normal Inulin clearance was 57 ml/min



Fig 1 Aortogram Renal artery stenosis and tumor mass (arrows)

The chest X rays and ECGs were normal A radiorenogram demonstrated normal renal function on both sides A second evaluation of the aortography films reconfirmed the diagnosis of right renal artery stenosis Moreover a 2 cm nodule was seen opacifying above the renal arteries close to the aorta This was interpreted as consistent with a tumor mass possibly a pheo- (Fig 1) A retrograde phlebography of the adrenal vein performed to elucidate the nature of the mass proved to be of no additional diagnostic value

Renal vein renin studies were carried out to evaluate the significance of the renal artery stenosis After catheterization of each renal vein under fluoroscopic control blood was drawn simultaneously from both sides Dihydralazine 0 2 mg/kg b wt was given i v to provoke a drop in BP and to stimulate the renin-angiotensin system Blood was again drawn 20 min thereafter for renin determination The results of the renin studies are given in Table I

The urinary excretion of catecholamines was highly

Table I *Renal venous plasma renin activity*

	Angiotensin/100 ml plasma in renal vein (ng)		
	Right	Left	Right/left ratio
Basal	880	790	1 1
After dihydralazine	2 280	720	3 1

Table II *Urinary excretion of catecholamines and vanillylmandelic acid (VMA) on different occasions during the preoperative investigation*

Adrenaline (μg/g creatinine)[a]	Noradrenaline (μg/g creatinine)[b]	VMA (mg/24 h)
12	191	4 2
21	178	4 4
77	135	6 4
33	158	15 0
22	136	6 0
		12 0
		3 8
		3 3
Upper normal limits 12	[b]34	7

elevated on several occasions (Table II) Only two of eight estimations of vanillymandelic acid (VMA) however were above normal

Because of the aortographic findings the results of renin studies and the elevated urinary excretion of catecholamines the patient was thought to have both a renal artery stenosis of functional significance and a pheochromocytoma

Surgery was carried out at the Department of Thoracic Surgery Karolinska Hospital on Feb 9 1971 under anesthesia with Leptanal[®] The patient was treated with phenoxybenzamine for four days before the operation

The aorta and the right renal artery were exposed through a thoraco-peritoneal incision and after dividing the diaphragm There was a spherical tumor approximately 2 cm in diameter close to the aorta above the renal arteries Removal of the tumor precipitated a sharp rise in BP which however was well controlled by i v phenoxybenzamine

The renal artery divided into two branches 1 cm distal from its junction with the aorta A pressure gradient of 23 mmHg was measured across the lesion in the superior branch in which a stenosis had been revealed on the aortogram There was no pressure gradient in the inferior vessel The stenosed artery was incised and a membranous narrowing was found A vein patch angioplasty was performed whereby the pressure gradient was eliminated

The postoperative clinical course was uncomplicated BP stabilized at 130/80 mmHg and the patient was discharged without drug therapy Microscopic examination of the tumor revealed a pheochromocytoma The histologic picture with growth of tumor tissue through the capsule was suspicious of malignancy Four months after the operation endogenous creatinine clearance had increased to 100 ml/min

The patient is free from symptoms three years after the operation The BP and urinary excretion of catecholamines are normal

DISCUSSION

Although in most cases it is possible to control high BP by the proper use of effective antihyperten

sive therapy and to considerably reduce the morbidity and mortality caused by hypertension it is at present not possible to cure hypertensive disease by medical therapy For this reason it is important to recognize the more common disorders resulting in hypertension that may be surgically remediable and to search for these disorders in at least young patients and in patients whose BP is not successfully controlled by medical therapy

In some rare cases several causes of hypertension amenable to surgical therapy have been present at the same time In the literature we have found 27 cases with co-existing pheochromocytoma and renal artery stenosis (2 5 7 10 11 13 14 16 18 19 20 21 22 24 26 27 29 30 31 33 35 40 41 42) In 16 cases the stenosis was considered secondary to compression by the tumor (5 11 13 14 16 19 23 27 29 30 33 41 42)

It was possible solely to excise the pheochromocytoma in 8 of these cases (11 13 16 19 30 41 42) One patient was treated with resection of the tumor and a graft angioplasty (33) and excision of the tumor and nephrectomy were performed in 6 patients (5 14 19 23 26 29)

Three patients with renal artery stenosis due to fibromuscular hyperplasia were treated with excision of the tumor and graft angioplasty (10 20 40) as was one patient with an arteriosclerotic stenosis (2) In 2 patients with renal artery stenosis due to an arteriosclerotic plaque excision of the tumor only was performed in the first instance When the hypertension persisted however nephrectomy was carried out at a second operation (18 24)

In one patient with co-existing pheochromocytoma and renal artery stenosis the artery was compressed by fibrous adhesions which were a consequence of the surgical treatment of a previous pheochromocytoma at that site This patient expired postoperatively (27)

Nephrectomy was performed in one patient with two pheochromocytomas but the nature of the artery stenosis could not be settled (31) and in 2 patients one of whom had a cervical pheochromocytoma only the tumor was excised and the renal artery was not inspected (22 35) In one patient with a suprarenal pheochromocytoma the artery stenosis was shown not to be due to organic causes (7)

The simultaneous occurrence of two surgically correctable causes of hypertension has in most cases raised considerable diagnostic difficulties In less than half of the 27 cases with co-existing pheochromocytoma and renal artery stenosis found in the literature both lesions had been suspected before the operation In one patient with angiographic evidence of renal artery stenosis the suspicion of a pheochromocytoma was even rejected because the simultaneous occurrence of pheochromocytoma and renal artery stenosis was felt to be too unlikely (19) even though the history was typical and the urinary excretion of VMA and catecholamines was elevated and a Regitine test was positive

In 9 cases a pheochromocytoma was found accidentally during surgery for renal artery stenosis (10 11 13 14 19 23 24 29) As the medical preparation of a patient with a pheochromocytoma for surgical removal of the tumor is very important and as it is essential to be able to anticipate cardiovascular complications this situation is most unfortunate In 5 of these 9 cases a major hypertensive crisis and/or serious ventricular arrhythmia complicated the surgery whereas severe elevation of the BP occurred in only one of the 13 patients with preoperative suspicion of a pheochromocytoma In the patient who died in close connection with the surgical procedure pheochromocytoma had been diagnosed preoperatively

The elusive nature and varied modes of presentation of the pheochromocytoma often make the diagnosis difficult In our patient several estimations of VMA were negative and the tumor was found unexpectedly as a result of re-evaluating the films taken during aortography This emphasizes the need to also search thoroughly for angiographic signs of a pheochromocytoma in circumstances where a renal artery stenosis is found as a probable cause of the hypertension

Conversely if a patient undergoes an operation for a firmly diagnosed pheochromocytoma resulting in the detection of an unsuspected renal artery stenosis the situation is probably less hazardous If hypertension persists postoperatively however the situation will be very complicated Search for additional tumors will be necessary and if the results are negative the hypertension will probably be considered residual to the prolonged excess of pressor amines As it is commonly stated that approximately 5–10 % of pheochromocytomas are malignant (12 15 32) this hazard too has to be considered A catheter angiography the most definite roentgenographic method for localization of a

suspected pheochromocytoma (44) is therefore of value also to exclude the co existence of a renal artery stenosis

The obvious question is did the renovascular lesion play a pathophysiologic part in our patient and in the patients with co-existing pheochromocytoma and renal artery stenosis reported earlier?

As the finding of a renal artery stenosis does not suffice to establish the diagnosis of renovascular hypertension the functional significance of the lesion must be tested Several studies have now confirmed the usefulness of determinations of renin activity in blood from each renal vein in evaluating patients with renal artery stenosis A renal vein renin ratio diseased side/normal side of more than 1 5/1 has been found to indicate renovascular hypertension potentially correctable by surgery (6 25 43)

In our patient the renin activity (3) in blood from both renal veins was markedly elevated on a normal sodium diet (100–150 mmol/day) (Table I) but there was no lateralization to the diseased side To avoid a false negative renal vein renin ratio the difference between the two sides can be augmented by stimulating the renin release in some way (6 8 17) In our patient the renin activity rose significantly in blood from the diseased side after i v dihydralazine administration but not from the contralateral side The renal vein renin ratio increased to 3 2/1 As a ratio of more than 1 5/1 even after stimulating the renin–angiotensin system seems to correlate well with results from surgery (17) we are convinced that the renal artery stenosis in this patient was of pathophysiologic significance In our opinion the hypertension would have persisted had the renovascular lesion not been treated surgically

Renal vein studies and/or split function studies were performed in only 2 of the 5 earlier cases with renal artery stenosis due to fibromuscular hyperplasia or arteriosclerosis In the case reported by McBride and Fitz (24) with an arteriosclerotic stenosis revealed by aortography the renal vein renin ratio (1 9/1) supported the diagnosis of renovascular hypertension A mass suspected of pheochromocytoma was found at laparotomy After resection of the tumor the BP fell markedly and exploration of the renal artery was therefore not undertaken Postoperatively however the BP remained elevated and for this reason nephrectomy was performed three months later BP then returned to normal ultimately proving the functional significance of the renovascular lesion In the case reported by Van Way et al (40) with a fibromuscular stenosis the renal vein renin activity lateralized markedly to the diseased side The hypertension of this patient was successfully treated with excision of the pheochromocytoma and a vein angioplasty

In some cases where the renal artery stenosis was considered secondary to compression by a pheochromocytoma positive split function tests suggested that the renovascular lesion contributed to the hypertension (19 29 30 33) Most of these patients were treated with removal of the tumor plus a graft angioplasty (33) or with nephrectomy (19 29) Two patients however were cured solely by excision of the pheochromocytoma (19 30) In one of these patients (30) the renin activity in peripheral blood was markedly elevated prior to the operation as was the urinary excretion of aldosterone After operation renin activity returned to values within the normal range The most natural explanation for this is that the mechanical compression of the renal artery was relieved by removal of the tumor and that the stimulation of the renin–angiotensin system was decreased when the blood flow to the artery was restored This argument cannot however be valid in the case reported by Mannhart et al (22) a young girl with a cervical pheochromocytoma plus a left renal artery stenosis revealed by aortography As the results of isotope nephrography and split renal function tests were within normal limits the diagnosis of renovascular hypertension was rejected The renin activity though was markedly elevated After excision of the pheochromocytoma the renin levels normalized Their case raises three questions Was the renin release in this patient stimulated by a high level of catecholamines in the blood? Was the presence of the renal artery stenosis a mere coincidence or did the high levels of catecholamines also cause the stenosis?

As to the first question animal studies in which noradrenaline adrenaline and isoprenaline have been infused into the renal artery at supraphysiologic concentrations have shown that renin release is stimulated by these hormones possibly by direct action (28 36 39) Data on the renin activity associated with a pheochromocytoma however are sparse and contradictory Some investigators have reported the renin activity to be normal (4 34) or even low (9) but in a recent study (38) 7 out of 8

patients with pheochromocytoma showed elevated plasma renin activity Maebashi et al (21) found in one case of pheochromocytoma that the urinary excretion of noradrenaline was clearly correlated to the elevation of plasma renin activity When discussing the second question as to whether elevated blood catecholamine levels might be the underlying cause of the stenosis it would of course have been of great interest to know whether the stenosis in the patient of Mannhart et al (22) persisted after removal of the cervical pheochromocytoma Aortography was unfortunately not repeated as part of the follow up One of the earlier cases however is instructive Ecoiffier et al (7) gave an account of a young girl who was investigated for hypertension An aortography performed under general anesthesia showed the existence of a suprarenal tumor on the right side and stenosis in the right and left renal the splenic and inferior mesenteric arteries The patient died of irreversible cardiac arrhythmia some hours after the aortography Radiologic and histologic studies of the left renal artery post mortem were entirely normal The renal artery stenosis was thus considered to have been non organic supposedly caused by high circulating levels of catecholamines This assumption receives support from experimental work on dogs in which i v infusion of adrenaline and noradrenaline caused localized and persistent spasm of the renal artery (1 14)

As for the three reported cases with pheochromocytoma and renal artery stenosis due to fibromuscular hyperplasia it has been suggested that catecholamines secreted by the tumor may have induced a contraction and functional stenosis in the artery (10 40) Long standing influence of hormones on the vessel may then have resulted in a secondary fibrous lesion and an irreversible organic stenosis No histologic examination of the artery was performed in our patient but the macroscopic appearance of the lesion was compatible with a fibromuscular stenosis thus the same pathophysiologic mechanism may be valid in this case

As in all four cases the hormone producing tumor was immediately adjacent to the artery it is possible that the arterial lesion was a result of local leakage of catecholamines rather than of elevated levels of the hormones in the circulation

Tumors often displace or invade vessels The blood flow may also be blocked by thrombotic masses secondary to tumors Evidence suggests that it is rare for an artery to be compressed mechanically by a tumor (14) This raises the question as to whether the reported cases of artery stenosis judged to be secondary to compression by a pheochromocytoma were in fact not due to compression of the tumor per se but to local diffusion of the catecholamines

REFERENCES

1 Abrams H L Boijsen E & Borgström K Effect of epinephrine on renal circulation angiographic observations Radiology 79 911 1962
2 Bethea M C Baltz H J Rogers R E & Weichert R F III Pheochromocytoma and renal artery South Med J 66 497 1973
3 Boucher R Veyrat R de Champlain J & Genest J New procedures for measurements of human plasma angiotensin and renin activity levels Can Med Assoc J 90 194 1964
4 Brown J J Davies D L Lever A F & Robertson J I S Plasma renin concentration in human hypertension II Renin in relation to aetiology Br Med J 2 1215 1965
5 Castelman B Scully R E & McNeely B U Case records of the Massachusetts General Hospital Case 19 1973 Presentation of case N Engl J Med 288 1010 1973
6 Cohen E L Rovner D R & Coun J W Postural augmentation of plasma renin activity importance in the diagnosis of renovascular hypertension JAMA 197 973 1966
7 Ecoiffier J Fournier A Leduc G & Plainfosse M -C Quelle signification peut-on accorder a la découverte arteriographique de stenoses au cours des pheochromocytomes Presse Med 78 2325 1970
8 Foster J H Dean R H Pinkerton J A & Rhamy R K Ten years experience with the surgical management of renovascular hypertension Ann Surg 177 755 1973
9 Fukuchi S & Katsushima I Measurement of plasma angiotensin II by radioimmunoassay Tohoku J Exp Med 103 185 1971
10 Garrett H E Scott R Jr Howell J F & De Bakey M E Pheochromocytoma and renal artery stenosis Surgical treatment of secondary hypertension a case report Arch Surg 90 97 1965
11 Georgakakis A K Chromaffin-cell tumours A review of 10 cases Br J Surg 58 895 1971
12 Harrison T S Gann D S Edis A J & Egdahl R H Surgical disorders of the adrenal gland Physiologic background and treatment p 112 Grune & Stratton New York 1975
13 Heberer G Engelking R & Eigler F W Diagnostische und therapeutische Besonderheiten bei einigen Hochdruckkranken mit Nierenarterienstenosen Dtsch Med Wochenschr 92 581 1967
14 Hudson P Clinicopathologic conference New Physician 18 308 1969

15 Huebner G D & Reed P A Secreting tumors of chromaffin tissue Ann Surg 158 216 1963
16 Ishibashi M Takeuchi A Yokoyama S Yamaji T Tsuchimochi T Tanaka T Kurihara H & Ikeda M Pheochromocytoma with renal artery stenosis and high plasma renin activity Jpn Heart J 16 741 1975
17 Kaneko Y Ikeota T & Takeda T Renin release during acute reduction of arterial pressure in normotensive subjects and patients with renovascular hypertension J Clin Invest 46 705 1967
18 Kaufman J J Marks L S & Smith R B Stenosis of the renal artery and coexistent lesions Surg Gynecol Obstet 139 59 1974
19 Kerzner M S Reeves J A DeNyse D & Claunch B C Pheochromocytoma with renal artery compression in an identical twin Arch Intern Med 121 91 1968
20 Kwan V W Hawkins L W & Lervick E R Pheochromocytoma and renal artery stenosis South Med J 63 114 1970
21 Maebashi M Miura Y Yoshinaga K & Sato K Plasma renin activity in pheochromocytoma Jpn Circ J 32 1427 1968
22 *Mannhart M Ludin H Veyrat R & Ziegler W* H Secondary hyperaldosteronism caused by cervical phaeochromocytoma cured by ablation of the tumor Helv Med Acta 35 479 1969–70
23 Marchal C & Sapelier P Hypertension artérielle sténose de l artère rénale et phéochromocytome Med Infant 74 341 1967
24 McBride J W & Fitz A Renal artery stenosis pheochromocytoma and erythrocytosis Am J Med 51 403 1971
25 Michelakis A M Foster J H Liddle G Rhamy R K Kuchel O & Gordon R D Measurement of renin in both renal veins—its use in diagnosis of renovascular hypertension Arch Intern Med 120 444 1967
26 Milliez P Bourel M Fritel D Tcherdakoff P & Samarcq P Pheochromocytome du pédicule vasculaire rénal Bull Soc Méd Hôp Paris 144 1027 1963
27 Naidich T P Sprayregen S Goldman A G & Siegelman S S Renal artery alterations associated with pheochromocytoma Angiology 23 488 1972
28 Reid I A Schrier R W & Earley L E Effect of beta adrenergic stimulation on renin release Adv Exp Med Biol 17 49 1972
29 Rosenheim M L Ross E J Wrong O M Hodson C J Davies D R & Smith J F Unilateral renal ischaemia due to compression of a renal artery by a phaeochromocytoma Am J Med 34 735 1963
30 Sato T Sakamoto S Maebashi M Suzuki C Furuyama T Yoshinaga K & Sudo K Hypertension due to combination of pheochromocytoma and unilateral renal ischemia by tumor compression Jpn Heart J 8 202 1967
31 Seringe P Plainfosse B Debrun G Allaneau C Bellaisch G Hinglais Mme Berry J P & Watchi J M Phéochromocytome bilatéral et sténose unilatérale de l artère rénale chez un enfant Opération Guérison Étude en microscopie électronique Ann Pédiat 44 714 1968
32 Sjoerdsma A Pheochromocytoma Current concepts of diagnosis and treatment Ann Intern Med 65 1302 1966
33 Straube K R & Hodges C V Pheochromocytoma causing renal hypertension Am J Roentgenol 98 ??? 1966
34 Streeten D H P Schletter F E Clift G V Stevenson C T & Dalakos T G Studies of the renin–angiotensin–aldosterone system in patients with hypertension and in normal subjects Am J Med 46 844 1969
35 Tinguely H Bopp P & Wettstein P Association phéochromocytome hyperplasie fibro-musculaire coincidence ou entité nosologique Radiol Clin (Basel) 35 311 1966
36 Vander A J Nature of the stimulus for renin secretion in anesthetized dogs Hypertension 13 126 1973
37 Vaughan E D Jr Buhler, F R Laragh J H *Sealey J E Baer L & Bard R H* Renovascular hypertension Renin measurements to indicate hypersecretion and contralateral suppression estimate renal plasma flow and score for surgical curability Am J Med 55 402, 1973
38 Vetter H Fischer N Bayer J M Schmitz T E Werning C & Vetter W Aldosteron Cortisol und Plasma-Renin Aktivitat beim Phaochromocytom Klin Wochenschr 52 719 1974
39 Wathen R L Kingsbury W S Stouder D A Schneider E G & Rostorfer H H Effects of infusion of catecholamines and angiotensin II on renin release in anesthetized dogs Am J Physiol 209 1012 1965
40 Van Way C W Michelakis A M Alper B J Hutcheson J K Rhamy R K & Scott H W Jr Renal vein renin studies in a patient with renal hilar pheochromocytoma and renal artery stenosis Ann Surg 172 212 1970
41 Weidmann P Siegenthaler W Ziegler W H Sulser H Endres P & Werning C Hypertension associated with tumors adjacent to renal arteries Am J Med 47 528 1969
42 Weiss M Muller H Kropelin T & Kluthe R Nierenarterienstenose und Phäochromozytom Verh Dtsch Ges Inn Med 79 812 1973
43 Winer B M Lubbe W F Simon M & Williams J A Renin in the diagnosis of renovascular hypertension JAMA 202 121 1967
44 Zelch J V Meaney T F & Belhobek G H Radiologic approach to the patient with suspected pheochromocytoma Radiology 111 279 1974

Acta Med Scand 202 237–240 1977

Anticoagulant Treatment in Sarcoidosis

Eva Hedfors

From the Departments of Thoracic Medicine and Medicine Karolinska Hospital Stockholm Sweden

ABSTRACT The treatment of sarcoidosis has so far been disappointing This paper presents a patient in whom persistent disappearance of the pulmonary infiltrations was observed during anticoagulant treatment for venous thrombosis A possible mechanism for a beneficial effect of anticoagulant treatment in sarcoidosis is discussed

In most cases of sarcoidosis the prognosis is favourable with complete recovery within two years during which period the patient has no symptoms (14) However there is always a risk that patients with long standing disease eventually develop progressive pulmonary fibrosis So far no treatment has been available to inhibit this process Corticosteroids which are often used in these cases or instituted on the presence of respiratory symptoms usually lead to immediate relief and a regression of the pulmonary infiltrations However in most cases the effect has been only suppressive or temporary without affecting the long term outcome of the disease (8 10)

The present case might indicate an alternative form of management

CASE REPORT

A 33 year-old woman suffered from erythema nodosum at the age of 24 Six years later she began to experience shortness of breath during exercise A chest X ray in 1973 disclosed enlarged bilateral hilar glands and extensive fine nodular pulmonary infiltrations compatible with sarcoidosis stage II (Fig 1) Physical examination and laboratory data were normal The tuberculin skin test was negative at 2 TU Repeated cultures for Mycobacteria were negative A spirometry test revealed a slight ventilatory restriction without signs of obstruction The patient refused histopathological confirmation However the diagnosis of sarcoidosis was established by clinical data in combination with the chest X ray

The pulmonary infiltrations were unchanged during an observation period of six months when corticosteroids (flubensilon) were instituted (Celestona® Schering 1 5 mg daily) After two months the treatment was discontinued due to an allergic reaction with urticaria As the patient wished to complete the corticosteroid treatment despite moderate weight gain and slight cushingoid changes it was reinstituted after two months with successively diminishing doses of prednisolone (Prednisolon® ACO 15–5 mg daily) during another four months No further allergic reactions were noted During the treatment the respiratory symptoms improved Regression of the pulmonary infiltrations was also noted in April 1974 (Fig 2)

In Dec 1974 five months after completed corticosteroid therapy the patient developed physical signs of a deep venous thrombosis in the left leg The diagnosis was confirmed by plethysmography Oral contraceptives were considered a contributing factor and were omitted Anticoagulant treatment was started with heparin followed by warfarin for six months without any side effects Complete circulatory restitution was noted within two months A chest X ray taken when anticoagulant treatment started revealed reappearance of the pulmonary infiltrates (Fig 3) Ten months later and four months after completed anticoagulant treatment the chest X ray was considered normal This was also the case in April 1976 when the patient was also free from respiratory symptoms (Fig 4)

DISCUSSION

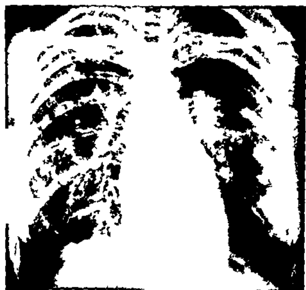
The outcome of sarcoidosis is never predictable Although rare complete spontaneous recovery may be seen even after eight years as in the present case Corticosteroid treatment cannot in the individual case be excluded as a contributing or initiating factor However a beneficial effect of anticoagulant treatment in sarcoidosis must be considered and possible mechanisms will be discussed



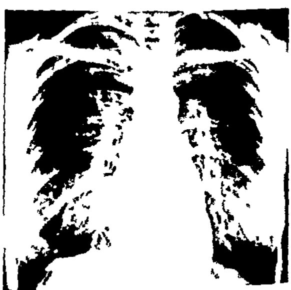
F g 1 Chest X ray on the in t al adm ss on in 1973 show ng pulmonary inf ltrat ons



F g 2 Regress on of pulmonary inf ltrat ons during cort costero d treatment (April 1974)



F g 3 Reappearance of pulmonary nf ltrat ons in Dec 1974 f ve months after completed cort costero d treat ment



F g 4 Normal chest X ray in April 1976 11 mon hs after completed ant coagulant treatment

The etiology of sarco dosis is st ll unknown However the histopatholog cal p cture with exten sive epithel oid cell granuloma formations in the affected organs may indicate that the pathogenetic mechanism of the disease is a delayed type hypersensitivity react on to an unknown antigen (9 14) The impaired T cell funct on which is characteristically found in the disease does not speak against a delayed hypersensitivity mechanism It is a non specif c phenomenon and should be looked upon as a consequence of the disease rather than its cause (9)

The finding of c rculat ng immune complexes in sera from patients with sarcoidosis of acute onset during the initial phase of the d sease m ght how ever imply that the granulomas are in t ated by a humoral immune mechanism (9) Depos ts of im munoglobul ns and complement (C3) have al o

been found in addition to fibrinogen in the affected organs (16) However the rare finding of sarcoidosis in agammaglobulinaemic patients speaks against a humoral mechanism as *the sole* cause (14)

The effect of anticoagulants on delayed hypersensitivity (DH) reactions was shown initially by Nelson (11 12) After intraperitoneal intravenous or subcutaneous injection of antigen into guinea pigs primed for DH peritoneal macrophages adhere to each other and to the peritoneal serosal cells The reaction which is called macrophage disappearance reaction does not occur in normal animals or in animals with pure Arthus hypersensitivity (12) The reaction in DH primed animals is inhibited by warfarin and heparin In contrast the effect of cortisone acetate is slight and largely accounted for by the decrease in the number of macrophages initially present in the exudates (11)

Furthermore in BCG primed animals treated with heparin or warfarin the tuberculin skin reaction after 48 hours is smaller than in controls Treatment with cortisone produces slightly less inhibition of the reactions whereas the combined treatment with cortisone and anticoagulants produces the most marked inhibition The effect is due to more marked inhibition of macrophage accumulation at the skin test sites by anticoagulants plus cortisone than by cortisone alone The inhibitory effect of anticoagulants on macrophage accumulation seems to be dependent on the presence of antigen (11) whereas the cortisone seems to reduce the number of macrophages available (11 15)

The suggestion that macrophage adherence may be mediated by the activation of the clotting system is further substantiated by the finding that fibrin binds to the surface of guinea pig peritoneal macrophages to a still unidentified receptor which is distinct from that for cytophilic IgG and C3 (4) Moreover recent studies have demonstrated that fibrin accumulation is a prominent and consistent feature of delayed hypersensitivity skin reactions in man and responsible for their characteristic induration (5 6) Whether the infiltration is brought about by soluble factors (lymphokines) from activated T lymphocytes has been discussed (4)

Anticoagulants have already been widely used alone or in combination with antiaggregates (e g dipyramidole acetylsalicylic acid) in the treatment of clinical conditions with immunoinflammatory tissue damage where the role of thrombosis and fibrin deposition is evident A beneficial effect has been reported in kidney and heart allografts (2 7) proliferative glomerulonephritis (3) systemic lupus (13) and thrombotic microangiopathy (1)

So far the treatment seems not to have been used in pure granulomatous conditions If this is done in sarcoidosis it must be remembered that the prognosis is usually favourable without any treatment Furthermore the initial granulomatous reaction should probably be considered as a defense mechanism aiming at limiting the spread of the initiating agent However it is equally evident that in certain cases especially of long duration the reaction may be deleterious to the patient In such situations warfarin treatment might be beneficial

REFERENCES

1 Amir J & Krauss S Treatment of thrombotic thrombocytopenic purpura with antiplatelet drugs Blood 42 27 1973

2 Busch G J Kobayaski K Kollenberg N K Birtch A G & Colman R W Hyperacute renal allograft rejection in the primate Intrarenal effects of heparin and associated net release of factor VIII activity and kallikrein activation Am J Pathol 80 1 1975

3 Clarkson A McDonald M Cash J & Robson J Modification by drugs of urinary fibrin fibrinogen degradation products of glomerulonephritis Br Med J 2 255 1972

4 Colvin R & Dvorak H Fibrinogen/fibrin on the surface of macrophages Detection distribution binding requirements and possible role in macrophage adherence phenomena J Exp Med 142 1377 1975

5 — Role of clotting system in cell mediated hypersensitivity II Kinetics of fibrinogen/fibrin accumulation and vascular permeability changes in tuberculin and cutaneous basophil hypersensitivity reactions J Immunol 114 377 1975

6 Dvorak H & Mihm M Basophilic leucocytes in allergic contact dermatitis J Exp Med 135 235 1972

7 Griep R Wexler L Stinson E Dong E & Shumway N Coronary arteriography following cardiac transplantation JAMA 221 147 1972

8 Hedfors E Influence of steroid treatment on lymphocyte response in sarcoidosis Clin Immunol Immunopathol 4 96 1975

9 — Immunological aspects of sarcoidosis Scand J Respir Dis 56 1 1975

10 Israel H Fouts D & Beggs R A controlled trial of prednisone treatment of sarcoidosis Am Rev Respir Dis 107 609 1973

11 Nelson D The effects of anticoagulants and other drugs on cellular and cutaneous reactions to antigens in guinea pigs with delayed type hypersensitivity Immunology 9 219 1965

12 Nelson D & Boyden S The loss of macrophages from peritoneal exudates following the injection

of antigens into guinea pigs with delayed type hypersensitivity Immunology 6 264 1963
13 Ponticelli A Imbasciati E Brancaccio D Tarantino A & Rivolta E Acute renal failure in systemic lupus erythematosous Br Med J 3 716 1974
14 Scadding J Sarcoidosis Eyre and Spottiswoode London 1967
15 Thompson J & van Furth R The effect of glucocorticoids on the proliferation and kinetics of promonocytes and monocytes of the bone marrow J Exp Med 137 10 1973
16 Wanstrup J & Elling P Immunochemistry of sarcoidosis Acta Pathol Microbiol Scand 73 37 1968

ANNOUNCEMENTS

One million Swiss francs from the Cilag Chemie Foundation Over the last five years the Cilag Chemie Foundation for Therapeutic Research has allotted at international level almost one million Swiss francs for pharmacological research The aim of the Foundation instituted in 1970 by Cilag Chemie AG Schaffhausen a Swiss pharmaceutical company is to promote research and investigations in therapy The Board of Trustees is composed of renowned professors from Switzerland the German Federal Republic Scandinavia and Great Britain

From 1972 to 1976 funds totalling Sfr 752000 were awarded to medico-scientific institutes and clinics for pharmacological research In 1977 the Foundation will disburse a further Sfr 214000 This year support will be given to research projects in internal medicine (studies of heart kidney and intestinal diseases as well as allergies) and in gynaecology

Announcements concerning requests for application forms are published in the first months of every year in the following medical journals British Journal of Clinical Pharmacology European Journal of Clinical Pharmacology European Journal of Clinical Investigation

The VIIth International Congress of Nephrology will take place in Montreal Canada June 18–23 1978 The scientific program will include plenary sessions symposia workshops free communications French and English are the two official languages Sponsored by the International Society of Nephrology the Canadian Society of Nephrology Quebec s Association of Nephrology and the Kidney Foundation of Canada

Organizing committee President G Lemieux Vice president J Dirks Treasurer J G Mongeau Secretary general M Bergeron

Information Dr M Bergeron Secretary general Université de Montréal Département de Physiologie C P 6208 Succ A Montréal Québec Canada H3C 3T8

XIII World Congress on Diseases of the Chest sponsored by the International Academy of Chest Physicians and Surgeons affiliated with the American College of Chest Physicians will be held in Kyoto Japan July 2–7 1978 Program subjects include Reconstruction of the trachea and bronchial tree Current trends in cardiothoracic surgery Early diagnosis of esophageal cancer Update on treatment of tuberculosis Ischemic coronary disease Diagnosis and management of acute respiratory failure Motion picture sessions Exhibits Original investigations

Information Committee on Scientific Program XIII World Congress on Diseases of the Chest American College of Chest Physicians 911 Busse Highway Park Ridge Illinois 60068 USA

XIV Congress of the International Society of Internal Medicine will be held in Rome Italy Oct 15–19 1978

Four preliminary sessions are planned for symposia and lectures in the mornings and in the afternoons Further details will be published later

Acta Med Scand 202 241–251 1977

Recording of Drug Prescriptions in the County of Jamtland, Sweden

Pattern of Drug Usage in 16600 Individuals during 1970–75

G Boethius

From the Department of Medicine Ostersund Hospital Ostersund and the Department of Clinical Pharmacology Karolinska Institutet Huddinge Hospital Huddinge Sweden

ABSTRACT The pattern of drug usage as obtained from a continuous recording of prescriptions in the county of Jamtland, Sweden is described Of 16600 persons monitored, about 60% purchase prescription drugs every year Considering all drugs, 55% of the patients make 4 purchases or less and 21% 10 purchases or more (mean 6 2 for men, 7 1 for women) A small portion (4%) of the patients accounts for 20% of the total prescriptions and 21% of the total costs The six largest pharmacologic groups of drugs antimicrobials (prescribed to 21% of the population) analgesics (19%) psychotropics (18%), drugs used in respiratory diseases (15%) cardiovascular (14%) and gastrointestinal drugs (13%), constitute 67% of total purchases and 73% of total costs Evidence for the representative nature of the recorded data is presented Women dominate in all age groups except the youngest and in all main groups of drugs Although exposition to drugs generally increases markedly with age, prescriptions of antiallergic and ear, nose and throat drugs decrease with increasing age As the indication for the prescription is not recorded the relationship between prescription and incidence of disease is clear only for drugs used for a single indication e g antidiabetics With the exception of drugs such as antidiabetics, cardiac glycosides and antihypertensives, there is a marked fall in prescribing during the summer Every year 50% of the patients receive prescriptions from one physician only, while 1% obtain drugs from 7 or more doctors Physicians outside the county hospital (30% of total) issue 74% of the total prescriptions The total number of prescribed drug specialities has been little affected by the recommendations from the Drug Committee of the county but the number of recommended drugs which were prescribed by many physicians increased from 1972 to 1975 In the case of serious adverse drug reactions the data will provide information on the number of patients exposed to the drug, the duration of exposure and the number of physicians who prescribe the drug At the time of its withdrawal from the market practolol had been prescribed to 112 of the monitored individuals, who could have been reached for follow up if necessary Seven of the 33 physicians prescribing the drug issued their first prescription after the manufacturer s preliminary warning about side effects

Since 1970 outpatient prescriptions dispensed to 1/7 or 16600 of the inhabitants in the county of Jamtland Sweden have been continuously recorded (12) Analysis of the data obtained so far includes the pattern of drug usage in various groups of patients or individuals such as pregnant women and blood donors (6 7 8 9) The longitudinal design of the project has also permitted follow up studies of drug usage (11)

The purpose of this paper is to further illustrate the kinds of data which can be derived from this project Emphasis will be put on information which cannot be obtained from drug sales statistics or prescription studies based on non identifiable samples

MATERIAL AND METHODS

The basic methodology used to obtain prescription data as well as the geographical conditions and health care structure in the county of Jämtland have been presented elsewhere (12) Since 1970 about 16600 persons have been monitored This corresponds to all individuals born on the same four days in each month Data from prescriptions filled at the pharmacies are continually compiled in drug lists These contain the patient s identity number (denoting e g age and sex) the year and week the drug was

Table I *Distribution of main pharmacologic drug groups in 1974 by percentages of population with at least one purchase of total purchases of total cost*

Drugs	% of population	% of total purchases	% of total cost
Antimicrobial	20 9	8 3	13 0
Analgesic	19 0	11 7	10 6
Psychotropic	18 0	15 9	11 6
Respiratory	15 0	8 1	6 0
Cardiovascular	14 4	14 3	24 2
Gastrointestinal	13 4	8 2	7 2
Vitamins	11 7	4 8	3 4
Antiallergic	11 5	4 1	2 7
Ear nose and throat	10 4	3 2	1 1
Dermatologic	9 1	3 8	3 8
Gynecologic	7 1	3 1	3 3
Hematologic	6 8	2 4	2 5
Ophthalmologic	5 2	2 9	1 6
Endocrinologic	4 1	3 9	5 9
Electrolytes	3 4	1 6	1 1
Other	3 3	3 7	2 0
Total	61 6	100 0	100 0

purchased the prescribing doctor the total amount and dosage of the drug and the type of prescription record The indication for the prescription is not available Drugs obtained without prescription are not recorded

Variables studied in this survey include *Number of individuals* (patients) Monitored population in 1970 8 471 men 8 071 women in 1974 8 535 men 8 194 women In the 5 year period 1970–74 10 213 men and 9 750 women included in the study resided in the county (12) *Number of purchases* Dispensed or refilled prescriptions *Number of defined daily doses* (DDD) (5) DDD is the agreed average dose used for the main indication of a drug e g 40 IU insulin 1 5 g tolbutamide etc (1) *Number of specialities* The about 1 800 drugs registered in Sweden have different formulations (tablets solution etc) and strength making up a total of 2 844 specialities in 1970 and 2 618 in 1974 *Number of physicians* The prescribing doctor is coded not as an individual but as the holder of a post e g resident in the Department of Medicine Since there is often more than one post of a kind at each clinic the number of prescribing physicians may be underestimated *Cost calculated as wholesale price at the pharmacy The true cost for the patient and for society has not been considered in this study* 1 Sw cr ≈0 25 US$ *Seasonal variation in purchases* The χ^2 test was used to determine the significance of the variation between 13 4-week periods *Drug sales data* which include sales to hospitals (10–15%) have been obtained from Läkemedelsstatistik AB (Swedish Pharmaceutical Data)

The Drug Committee of the county of Jamtland originates from the hospital-centered committee formed in 1967 Since 1972 it covers the whole county and issues a drug formulary "Z läkemedel" which is revised yearly This formulary lists about 300 drugs recommended for use unless the conditions of the individual patient indicate otherwise

RESULTS

What drugs are prescribed?

The prescription of the main pharmacologic groups of drugs is presented in three ways in Table I Evidently the rank order of the individual drug groups varies with the mode of measurement The six largest groups are prescribed to 13–21% of the population and constitute 66 5% of total purchases and 72 6% of total costs In adults almost 3/4 of all drug purchases concern psychotropics cardiovascular drugs analgesics, antimicrobials drugs used in respiratory diseases and gastrointestinal drugs (Fig 1) In young ages drugs used in ear nose and throat disorders allergy and gynecologic conditions are quantitatively important

To whom are drugs prescribed?

Age and sex distributions of persons obtaining drugs are presented in Fig 2 In general drug exposition increases markedly with age (Fig 2*a*) In some drug groups like antiallergic and ear nose and throat drugs, there is a decrease with increasing age (Fig 2*b*) Other groups such as dermatologic drugs respiratory tract agents and antimicrobial drugs are rather evenly prescribed in all ages (Fig 2*c*) The majority of drugs though increase in use with advancing age (Fig 2*d*) Women dominate in

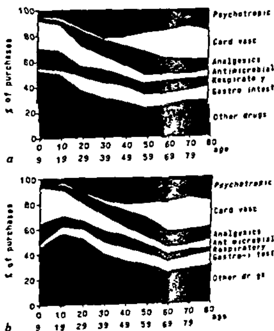


Fig 1 Distribution of drug groups within each age interval in 1974 (*a*) Men (*b*) women

Fig 2 Proportion of population with drug purchases in 1974 (*a*) All drugs (*b*) drugs decreasing with age (*c*) drugs rather evenly prescribed in all *d*) drugs increasing with age

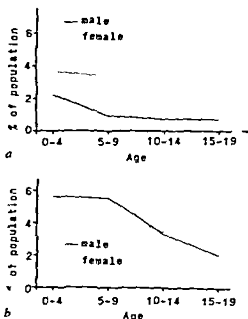


Fig 3 Proportion of population aged 0–19 with purchases of (*a*) drugs used in urinary tract infection and (*b*) psychotropic drugs in 1974

all age groups except the youngest and in all main groups of drugs

In young people purchases of some drug groups show a marked increase in females 15–19 years of age (Fig 3) The rise in psychotropic drugs is mainly attributed to benzodiazepines and combinations of barbiturates and belladonna alkaloids

In drug groups used mainly for a single indication the frequency figures may give an idea of how common a disease condition or symptom is in the population at various ages (Fig 4) Due to the longitudinal design of the study and the monitoring of defined individuals it is possible to state not only how many persons are exposed to drugs in one year but also to find out the proportion of the population exposed during longer periods In most drug groups the 5 year cumulative number of exposed individuals is 2–3 times greater than the number in a single year (Fig 5)

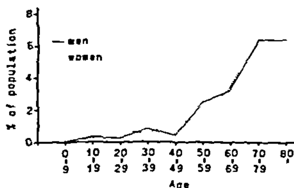


Fig 4 Proportion of population with purchases of antidiabetic agents in 1974

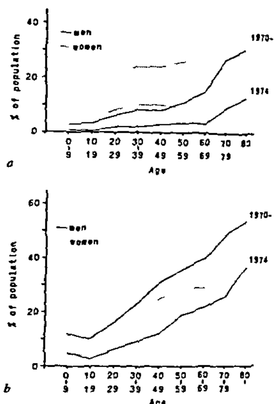


Fig 5 Proportion of population with purchases of (*a*) drugs used in urinary tract infection and (*b*) psychotropic drugs in 1974 and in the 5-year period 1970–74

How many drug purchases are made?

The cumulative number of prescriptions dispensed per individual in one year (1974) is seen in Table II Considering all drugs 55% of the patients made purchases or less while 21% made 10 purchases or more (mean 6 2 for men 7 1 for women) In a group like cardiovascular drugs representing mainly chronic treatment a greater number of prescriptions (men 4 3 women 3 9) are dispensed to the average patient compared with occasional short term treatment with antimicrobials (men 1 6 women 1 7 purchases/year) Psychotropic drugs represent an intermediate group with no difference between the sexes (men 3 7 women 3 6)

Table II *N mber of purchases per ind d al in 1974 Cumulat ve d str b t on (%)*

No of purchases	All drugs			Card ovascular drugs		Ant m crob al drugs		Psychotrop c drugs	
	Total	Men	Women	Men	Women	Men	Women	Men	Women
1	18 6	21 1	16 7	?8 ?	?8 6	67 5	61 9	4? I	41 8
?	34 0	37 4	31 2	4 4	46 5	87 9	84 8	59 0	59 5
3	45 5	48 8	4? 8	54 6	59 ?	94 5	93 6	70 1	69 3
4	54 5	57 7	51 8	65 5	69 6	96 8	96 3	75 9	78 6
(5–) 9	78 7	81 3	76 4	90 5	9? 5	99 8	99 9	91 8	9? 2
10–)14	88 9	90 1	87 9	97 6	98 5	99 9	100 0	96 8	96 7
15–)19	93 7	94 4	93 0	99 5	99 4	100 0		98 6	98 7
0–) 4	96 3	96 7	95 9	99 7	99 8			99 4	99 3
25–) 9	97 8	97 9	97 7	99 9	99 9			99.5	99.5
30–)91	100 0	100 0	100 0	100 0	100 0			100 0	100 0
Average per person	6 7	6 2	7 1	4 3	3 9	1 6	1 7	3 7	3 6

I gh cons mers

n 1974 3 7% of the pat ents obtained more than 24 rescript on drugs The purchases of th s group of at ents represented 20% of the total amount and 21% of the total cost of drug treatment in the coun y Some characterist cs of th s group are given in Table III Psychotrop c drugs seem to compose a greater part (?3%) of their purchases compared to he population as a whole (16%)

Who prescr bes drugs?

Every year 50% of the pat ents receive prescrip ons from one phys c an only wh le 1% visit 7 doctors or more (Table IV) The average number of phys c ans per male was 1 8 per female 2 0 in 1974 The pattern for some drug groups representing hronic and/or acute treatment is also seen in Table IV

Hosp tal phys cians making up 70% of the total number of physicians in the county were responsi ble for 26% of the outpat ent prescriptions in 1975 Phys cians outs de the hosp tal i e district phys cians (22%) and private pract tioners (8% of total doctors) issued 5?% and 22% of the prescript ons respectively The d stribut on with n some drug groups between the physic an categories is shown in Table V

What doses are prescr bed?

A study of the variab l ty of prescribed da ly doses and dosage intervals in relat on to pharmacokinet c princ ples is publ shed elsewhere (10)

The cost of prescr b ng

The cost per prescript on d ffers somewhat with age and sex (Fig 6) As the number of prescriptions per ind v dual increases with age the ndividual cost also shows a maximum in older age

The prescrib ng cost differs somewhat between phys c an categories (Table VI) In prescript ons issued by hosp tal physicians espec ally those n surgery and pulmonary med c ne there is a pronounced sex difference male purchases be ng more expensive than female

Seasonal var at on in prescrib ng

The seasonal variat on in prescribing arises mainly from the morb d ty pattern and vacat on act vities Cons dering all drugs there is a marked fall in the

Table III *Characterist cs of ind v duals w th more than 24 prescr pt on drug purchases in 1974*

	Ind viduals with > 4 purchases		All purchas ng ndiv duals
	N	% of total	
No of nd v duals	384	3 7	10 305
No of purchases	13 493	19 6	68 785
Cost (Sw cr)			
Total	388 339	?0 5	1 894 34?
Per purchase	29		8
Per person	1 011		184
Females (%)	60		55
Aged 60 years or more (%)	64		?9
Average no of pre scrib ng phys c ans	4 3		1 9

led 5 and 20

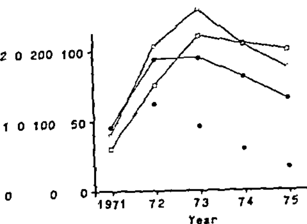


Fig 10 Prescribing of clonidine (Catapresan®) measured in number of defined daily doses (□) number of purchases (○) and number of patients on drug (●) =New patients on drug every year

General effect of local recommendations on prescribing

The total number of prescribed drug specialities has been affected little by the recommendations from the Drug Committee, a decline being seen from 728 in 1970 to 705 in 1974 However in most pharmacologic groups the number of recommended drugs being prescribed by many physicians increased from 1972 to 1975 Still 45% of all prescribed cardiovascular specialities are used by 1–4 physicians only (Table VII) Compliance with the recommendations differs with drug group and physician category (Fig 12) In the cardiac glycoside group a trend towards uniformity in prescribing is seen among all physicians regardless of

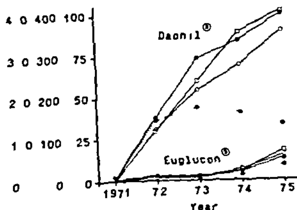


Fig 11 Prescribing of two glibenclamide preparations (Daonil® Euglucon®) measured in number of defined daily doses number of purchases and number of patients on drug Symbols as in Fig 10

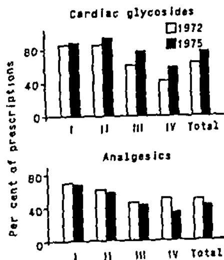


Fig 12 Percentage of prescriptions issued in compliance with the recommendations of the Drug Committee in 1972 and 1975 Physician category *I*=internal medicine *II*=all hospital physicians *III*=district physicians *IV*=private practitioners

level of compliance With regard to analgesic drugs on the other hand with 10 times as many specialities as in the cardiac glycoside group there is a small decrease in the proportion of prescriptions of recommended preparations A lower level of compliance among physicians outside the hospital is seen in most pharmacologic groups

Effect of warnings of side effects

The fall in the use of rauwolfia derivatives in 1975 illustrated in Fig 9 was most probably related to the reports in 1974 on a suspected link between long term reserpine use (in hypertension) and breast cancer In Oct 1974 a recommendation was issued by the Swedish health authorities to restrict the use of these drugs

A more detailed example is given in Fig 13

Table VII *Percentage of all prescribed cardiovascular specialities being used by various numbers of physicians in 1972 and 1975*

No of prescribing physicians	% of prescribed drug specialities	
	1972	1975
1–4	45	45
5–19	41	28
20–(112)	14	27
Total	100	100

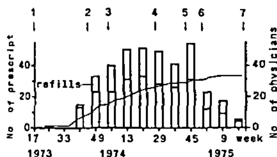


Fig 13 Number of dispensed prescriptions of practolol (bars) and cumulated number of prescribing physicians (curve) Arrows indicate data for registration of drug (*1*) drug information in county by manufacturer (*2*) evaluation of drug in Swedish medical press (*3*) letters to physicians from manufacturer about adverse effects (*4–5*) information about adverse effects and recommendations from Swedish Adverse Drug Reaction Committee (*6*) withdrawal of drug (for oral use) (*7*)

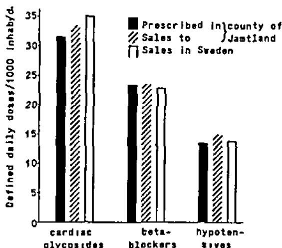


Fig 14 Comparison between prescription data and sales data in 1974 Sales in Sweden figures include sales to hospitals (10–15%)

Practolol a cardioselective β receptor blocking drug was registered in April 1973 and four months later prescribing started in the county Coinciding with the first reports to physicians about ocular and cutaneous side effects the prescribing curve level led off and after recommendations to restrict its usage fell drastically There was no significant increase in the proportion of refill dispensations after the warnings and thus no indication that the patients themselves were mainly responsible for continuing treatment On the contrary 7 of the 33 physicians prescribing practolol issued their first prescription after the preliminary warning from the manufacturer Altogether 112 patients (850 when extrapolated to the whole county population) were exposed to the drug before it was withdrawn 2 years after its introduction

DISCUSSION

An important question is whether the county of Jamtland is representative of the country as a whole as far as drug prescription is concerned For several important drug groups there is good agreement between the prescription data in the county on the one hand and wholesale figures for the whole of Sweden as well as for the county on the other (Fig 14) Within individual drug groups differences are found depending on local traditions in prescribing (Table VIII)

Data from a 1/288 sample of all prescriptions in Sweden (13) also confirm the representative nature of the Jamtland figures as regards the distribution between the main pharmacologic groups The level of prescribing in the county seems to be slightly lower than in the country as a whole 4 2 against 4 7 prescriptions per inhabitant

In a similar project in Tierp a more southern area of Sweden (4) a slightly higher prescription rate than in Jamtland was found in 1972 for respiratory tract agents cardiovascular drugs vitamins and psychotropics while the reverse was observed for antihistamines In 1975 the differences were smaller with the exception of cardiovascular drugs (14)

Prescribing in the county of Jamtland thus seems to be comparable to that in the country as a whole As has been shown above and in other studies (11) drug sales figures expressed in DDD often agree satisfactorily with variables such as number of purchases or number of tablets prescribed In other instances knowledge of the individual use of drugs

Table VIII *Number of defined daily doses (DDD) of β-receptor blocking agents sold in Jamtland and the whole of Sweden in 1974*

Drug	DDD (mg)	Jamtland No	Jamtland %	Sweden No	Sweden %
Alprenolol	300	5 7	24 2	10 1	44 1
Propranolol	160	15 1	64 3	11 0	48 1
Practolol	300	2 6	11 1	1 4	6 1
Pindolol	15	0 1	0 4	0 4	1 7
Total		*23 5*	*100 0*	*22 9*	*100 0*

in a defined population is desirable or necessary A most important measurement for medical purposes is the proportion of the population exposed to certain drugs

There have been small changes from 1970 to 1974 in the prescription of the main pharmacologic groups Psychotropic drugs have decreased their share with a corresponding increase of 2–3% in cardiovascular and antihistamine drugs

Whether the level of prescribing is rational cannot be judged from these prescription figures alone Other investigations based on our data illustrate this fact (6 11) Moreover, it is not known to what extent the drugs obtained were actually ingested

As the indication for prescribing is not recorded in this study the relationship between prescription and incidence of disease may be complex Exceptions are drugs used solely for a single indication e g antidiabetics although not all diabetics are *treated with drugs The prevalence of hypertensive* patients on drug treatment on the other hand is more difficult to state from our data To the number of patients using specific antihypertensive drugs such as methyldopa or hydralazine should be added an unknown proportion of patients on diuretics and β receptor blocking agents which are used on other indications as well (6) In a Swedish population study in 1968–69 on 1462 women aged 38–60 years (2) 64% of those treated with diuretics stated that they were prescribed for hypertension (3) With regard to β receptor blocking drugs in Jamtland it may be noted that 30–40% of the patients are on doses usually used in hypertension

Even with these limitations the rough prevalence figures obtained on morbidity may be of value for the planning of health services such as the organization of specialized (outpatient) clinics for hypertension or diabetes or setting up new methods for drug analysis in body fluids Other potential uses of the prescription data have been discussed elsewhere (12)

The prescribing pattern of different physician categories naturally reflects the morbidity pattern of the diseases they are treating The sex ratio and mean age (44–46 years) of their patients do not differ significantly between hospital physicians district physicians and private practitioners The more expensive prescriptions to men issued by physicians in e g internal medicine pulmonary medicine and surgery, are all attributed to the age groups above 40 years Cardiovascular drugs to these men are more expensive than drugs prescribed to women The prescribing of expensive antituberculous drugs almost exclusively to men and the surgeons prescriptions of hormones to patients with prostatic carcinoma explain the sex difference in drug cost.

The lower level of compliance with local drug committee recommendations among physicians outside the hospital is not unexpected since the drug committee up till recent years was active mainly among hospital physicians Knowledge of the prescribing habits in the county is fundamental for the local drug committee in several ways Firstly, the local tradition in prescribing will influence the choice between two or more preparations otherwise considered equivalent The presciption data will tell whether one doctor is responsible for most of the prescriptions or several doctors occasionally prescribe the drug in question Secondly the effects of the recommendations of the prescrib*ing pattern can be followed Thirdly knowledge* about local habits of prescribing may help to design relevant training programs in pharmacotherapeutics for physicians

In the case of serious adverse drug reactions our data will provide information on the number of patients exposed to the drug the duration of exposure and the number of doctors who prescribe the drug These variables may also be followed in an attempt to measure the effect of national information program on side effects The main reason for storing data on fully identifiable individuals for long periods is the desirability of being able to follow up potential late effects of any drug This should be in the interest of the individual as well as society

ACKNOWLEDGEMENTS

The project is sponsored by grants from the National Corporation of Swedish Pharmacies and the County Council of Jämtland

REFERENCES

1 Baksaas Aasen I Lunde P K M Halse M Halvorsen I K Skobba T J & Stromnes B Drug dose statistics List of defined doses for drugs registered in Norway Edited by Norsk Medisinaldepot, Oslo 1975
2 Bengtsson C Ischaemic heart disease in women Acta Med Scand (Suppl) 549 43 1973
3 — Personal communication
4 Berfenstam R & Smedby B Sjukvårdskonsumtion i Tierpsområdet 1971 (English summary) SPRI report 9 1974

Bergman U Elmes P Halse M Halvorsen T Hood H Lunde P K M Sjoqvist F Wade O L & Westerholm B The measurement of drug con sumption Drugs for diabetes in Northern Ireland Norway and Sweden Eur J Clin Pharmacol 8 83 1975

Boethius G The treatment of hypertension—an analysis of drug prescription data Acta Med Scand (Suppl) 602 120 1976

— Recording of drug prescriptions in the county of Jamtland Sweden II Drug exposure of pregnant women in relation to course and outcome of pregnan cy Eur J Clin Pharmacol In press 1977

— Recording of drug prescriptions in the county of Jamtland Sweden III Drugs presented for blood donors during a 5 years period Eur J Clin Pharmacol In press 1977

Boethius G Nordgren L & von Scheele C A five year survey of purchases of antacids anti cholinergic and psychotropic drugs J Psychosom Res In press 1977

10 Boethius G & Sjoqvist F Doses and dosage inter vals of drugs—clinical practice versus pharmacokine tic principles Clin Pharmacol Ther To be published

11 Boethius G & Westerholm B Purchases of hypno tics sedatives and minor tranquillizers among 2 566 individuals in the county of Jamtland Sweden—a 6 years follow up Acta Psychiatr Scand To be published

12 Boethius G & Wiman F Recording of drug pre scriptions in the county of Jamtland Sweden I Methodological aspects Eur J Clin Pharmacol In press 1977

13 Granzell A Kristoferson K & Wessling A Kontinuerlig kartlaggning av lakemedelskon sumtionen i Sverige Svensk Farm Tidskr 79 483 1975

14 Smedby B Personal communication

Acta Med Scand 202 253–255 1977

Bone Changes during Prednisone Treatment

Aage Deding Lars Tougaard Mogens Krogh Jensen and Paul Rødbro

From the Departments of Haematology and Clinical Physiology Ålborg Hospital Ålborg Denmark

ABSTRACT To evaluate changes in bone composition during treatment with corticosteroids, the bone mineral content (BMC, measured by photon absorptiometry) and the degree of bone mineralization (measured as the bone phosphorus/hydroxyproline ratio) were determined in 18 patients during prednisone treatment for haematological and connective tissue diseases The prednisone dose ranged from 12 to 51 mg/day (mean 27) The BMC decreased significantly (mean 2 5%) during the studied 12 weeks of treatment, but the change did not correlate significantly to the prednisone dose The degree of bone mineralization remained unchanged, indicating equal losses of mineral and of collagen in bone during prednisone treatment The changes correspond to a rapidly developing oesteoporotic state

One of the serious complications of steroid therapy is development of osteoporosis often aggravated by spontaneous bone fractures Bone rarefaction and reduced cortical thickness have been demonstrated in patients treated with glucocorticosteroids (2 8 10 11) The reported incidence of this complication has varied widely (8 10) depending on diagnostic criteria and selection of patients

Few investigators have followed the development of bone changes during steroid therapy (8 9) probably due to lack of suitable quantitative methods Radiology is not quantitative and cannot show bone changes until gross abnormalities are present Morphometric analysis of bone can be evaluated only by experts and requires large biopsies However two other methods have been developed from which quantitative information on bone composition can be obtained with little inconvenience to the patient The *degree of bone mineralization* can be estimated by measuring the phosphorus/hydroxyproline ratio (P/Hypro) in small bone biopsies (14 15) The *bone mineral content* (BMC) can be estimated by absorptiometry in the forearm (2 4 5, 13)

The purpose of the present study was to quantitate the changes in bone during prednisone treatment by measuring the BMC and the degree of bone mineralization

PATIENTS AND METHODS

Among the patients admitted to the Department of Haematology a consecutive series of 18 was selected for a 12 week study according to the following criteria. 1) Newly diagnosed haematological or connective tissue disorders requiring prednisone treatment 2) No previous treatment with steroids 3) No previous symptoms of bone diseases 4) Not immobilized for more than four days before the investigation There were 9 males and 9 females aged 47–78 years (mean 62) The diagnoses were chronic lymphocytic leukaemia ($n=8$) idiopathic thrombocytopenic purpura ($n=2$) polymyalgia rheumatica ($n=5$) temporal arteritis ($n=2$) and systemic lupus erythematosus ($n=1$)

The prednisone dose varied Patients with chronic lymphocytic leukaemia received prednisone in a dose of 30 mg/day for the first 6 weeks and 15 mg/day for the last 6 weeks besides chlorambucil The patients with connective tissue diseases and thrombocytopenic purpura received initially a high dose of prednisone (30–80 mg/day) thereafter a smaller maintenance dose (mean 32 mg/day) during the 12 weeks of investigation All patients showed normal physical activity during treatment which was started in hospital and continued on an outpatient basis

The BMC was measured in the forearm by photon absorptiometry (4,5) The bone biopsies for the P/Hypro analysis were obtained from the anterior superior iliac spine under local anaesthesia and divided for double determinations (intraindividual coefficient of variation 5 0%) The mean value was used in the study Since BMC and bone P/Hypro change with age (4 15) both indices were expressed as per cent of the corresponding normal mean The studied bone indices were measured before and after 12 weeks of prednisone treatment Plasma values of calcium phosphorus albumin and alkaline phosphatase were determined at the same time Values of plasma alkaline phosphatase were logarithmically

Table I *Summary of results (mean ± S D)*

	Reference values	Pre treatment values	Significance of dif ference	Change during treatment	Significance of change
Bone					
BMC (% of normal)	100±16 5	107 6±14 4	N S	−2 5±2 9	$p<0.01$
P/Hypro	100±7 0	98 1±6 0	N S	−1 6±4 6	N S
Plasma					
Calcium (mmol/l)	2 50±0 15	2 41±0 12	N S	0 06±0 17	N S
Phosphorus (mmol/l)	1 18±0 19	1 14±0 19	N S	−0 12±0 29	N S
Albumin (g/l)	45 0±6 5	36 9±6 3	N S	3 4±5 5	$p<0.0$?
Alkaline phosphatase (log μ/l)	2 07±0 19	2 17±0 57	N S	−0 13±0 16	$p<0.01$

N S = not significant

formed Normal values were either the reference values of our Department of Clinical Chemistry or have been published elsewhere (4 15) Changes from the pretreat ment values to the values after 12 weeks of treatment were evaluated by Student s *t* test for paired comparisons

RESULTS

None of the indices differed significantly from nor mal before treatment (Table I) The mean changes during prednisone treatment are given in Table I and individual values of bone indices before and after treatment are shown in Fig 1 BMC decreased *uniformly and significantly (mean 2 5%) during the* 12 weeks of prednisone treatment No significant change was found in bone P/Hypro The change in BMC was of the same order in the two sexes and in the group of patients with connective tissue dis eases compared with the group with haematological disorders with *no significant differences* The de crease in BMC did not correlate significantly to the mean dose of prednisone during treatment ($r=0.14$) or to the age

During the 12 weeks of treatment with pred nisone mean plasma albumin increased and mean plasma alkaline phosphatase decreased significant ly No significant changes were found in plasma calcium or plasma phosphorus

DISCUSSION

The altered calcium metabolism during prednisone treatment is due to changes in several organs in cluding the intestine (3 7 9) the kidney and bone (1 6 8 9, 11 12) But the reported changes in blood biochemistry during prednisone treatment differ from report to report (7 8 9 11) and cannot be considered fully clarified

The present study has demonstrated a significant decrease in BMC during prednisone treatment which could not be attributed to immobilization. The mean calcium loss during the 12 weeks was about 2 5% of the BMC in forearm which corre lates closely to total body calcium (4) Compared with the normal bone mineral loss with age of about

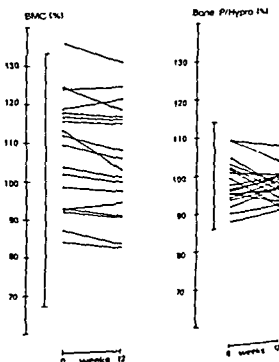


Fig 1 Individual values of bone mineral content (*BMC* % of normal) and bone phosphorus/hydroxyproline ratio (*P/Hypro* % of normal) before and after treatment with prednisone for 12 weeks Vertical bars indicate normal range (mean±2 S D)

0 75%/12 weeks (2 4) this is very high However the rate of calcium loss may well be particularly great in the initial phase of treatment and cannot be extrapolated directly to cover future calcium loss during prednisone treatment The high reproducibility of photon absorptiometry in the forearm makes this method valuable for longitudinal studies There is a considerable interindividual variation in BMC (4 5 13) which makes the method less valuable for studying small groups with single determinations This probably explains why Boyd et al (2) did not find significantly subnormal values of BMC in patients who had received therapeutic doses of prednisone for a long time The bone P/Hypro was unchanged during the 12 weeks of treatment This indicates an equal loss of bone mineral and collagen during prednisone treatment The findings correspond to a rapidly developing osteoporotic state since the physiological bone mineral loss is increased by a factor of 10 with no change in chemical bone composition as evaluated by the P/Hypro

REFERENCES

1 Atkins D & Peacock M A comparison of the effects of the calcitonins steroid hormones and thyroid hormones on the response of bone parathyroid hormone in tissue culture J Endocrinol 64 573 1975
2 Boyd R M Cameron E C McIntosh H W & Walker V R Measurement of bone mineral content in vivo using photon absorptiometry Can Med Assoc J 111 1201 1974
3 Carré M Ayigbedé O Miravet L & Rasmussen H The effects of prednisolone upon the metabolism and action of 25 hydroxy and 1 25-dihydroxyvitamin D3 Proc Natl Acad Sci USA 71 2996 1974
4 Christiansen C & Rødbro P Bone mineral content and estimated total body calcium in normal adults Scand J Clin Lab Invest 35 443 1975
5 Christiansen C Rødbro P & Jensen H Bone mineral content in the forearm measured by photon absorptiometry Principles and reliability Scand J Clin Lab Invest 35 323 1975
6 Cruess R L & Hong K C Effects of cortisone on collagenolytic activity in the rat Proc Soc Exp Biol Med 148 887 1975
7 DeLuca H F Calcium metabolism Acta Orthop Scand 46 284 1975
8 Gallagher J C Aaron J Horsman A Wilkinson R & Nordin B E C Corticosteroid osteoporosis Clin Endocrinol Metab vol 2 no 2 1973
9 Jee W S S Roberts W E Park H Z Julian G & Kramer M Interrelated effects of glucocorticoid and parathyroid hormone upon bone remodeling Excerpta Med Int Congr Ser 243 430 1972
10 Kjellstrand C M Side effects of steroids and their treatment Transplant Proc 7 123 1975
11 Leung K & Munck A Peripheral actions and glucocorticoid Annu Rev Physiol 37 245 1975
12 Lutwak L Metabolic and biochemical considerations of bone Ann Clin Lab Sci 5 185 1975
13 Shapiro J R Moore W T Jorgensen H Reid J Epps C H & Whedon D Oesteoporosis Evaluations of diagnosis and therapy Arch Intern Med 135 1975
14 Tougaard L The degree of mineralization in bone tissue The phosphorus/hydroxyproline ratio determined on small amounts of bone tissue Scand J Clin Lab Invest 32 351 1973
15 — The degree of mineralization in normal bone tissue estimated as the phosphorus to hydroxyproline ratio Scand J Clin Lab Invest 35 285 1975

Acta Med Scand 202 257–260 1977

Studies on Replacement and Suppressive Dosages of *l*-Thyroxine

G Nilsson U Pettersson K Levin and R Hughes

From the Department of Internal Medicine and the Laboratory of Clinical Chemistry Central Hospital Vasteras Sweden

ABSTRACT Serum thyrotropin (TSH) levels were studied in 55 hypothyroid patients in order to determine adequate replacement and suppression dosages of *l* thyroxine (T_4) In accordance with previous reports it was found that most patients had normal TSH levels and were clinically euthyroid at daily doses of 0 10–0 15 mg T_4 None of the patients required a dose exceeding 0 20 mg When the TSH levels normalized serum thyroxine and serum triiodothyronine also fell to levels within their normal ranges The effectiveness of various doses of T_4 in suppressing the temporary rise in serum TSH concentration normally induced by thyrotropin releasing hormone was examined in 57 patients treated with T_4 for atoxic goitre or after subtotal surgical removal of such a goitre The rise in TSH was not usually inhibited by a T_4 dose of less than 0 20 mg a finding which at least theoretically has implications for the adequate suppressive dose of T_4

In general thyroid hormone is administered to a patient for one or both of two reasons Firstly to replace failing endogenous production of thyroid hormone and secondly to suppress the patient s own thyroid hormone production via the pituitary thyroid negative feed back system in order to either prevent goitre formation or reduce goitre size

The dose of *l* thyroxine (T_4) required for these purposes is usually determined on a purely clinical basis Thus a dose is selected sufficient to remove symptoms of hypothyroidism or it is adjusted to be just below that giving rise to thyrotoxic symptoms such as excessive sweating or heart palpitations In practice the T_4 dose is probably often arbitrarily determined to attain a state somewhere between clear hypo- and hyperthyroidism when given for replacement purposes and somewhere below the level producing thyrotoxic symptoms when given for suppressive purposes

The control of T_4 medication by means of laboratory analysis has previously been very uncertain and of little practical value (9) This situation has been changed by the advent of radioimmunoassay for the determination of thyrotropin (TSH) Thus it may be assumed that an adequate replacement dose of T_4 in primary hypothyroidism should normalize the raised serum thyrotropin (S-TSH) level S TSH is probably the most sensitive index of peripheral thyroid hormone action illustrated by the fact that many patients with a slight S-TSH elevation show no clinical symptoms of hypothyroidism (»subclinical hypothyroidism») Recent reports have shown that most hypothyroid patients reach normal S TSH levels with daily T_4 doses of 0 10–0 15 mg (5 19) Only occasionally are doses of 0 20 mg required The dose necessary for normalizing S TSH has furthermore been reported to give serum thyroxine (S T_4) and serum triiodothyronine (S T_3) values within the generally accepted normal ranges although S T_4 tends to be slightly above and S-T_3 slightly below these normal values (19)

The suppressive thyroid hormone therapy aims at preventing thyroid stimulation by endogenous TSH secreted by the pituitary gland It is inadvisable to control the effectiveness of this suppression by estimating basal S TSH levels as methodological difficulties are encountered in the estimation of TSH in the lower normal range Instead the thyrotropin releasing hormone (TRH) test may provide valuable information (6 10) The hypothalamic hormone TRH normally causes a temporary

rise in S TSH but an effective suppressive T_4 therapy should inhibit the rise in S TSH normally induced by TRH

In order to determine the optimal dose level for T_4 replacement therapy we adjusted the T_4 doses in some hypothyroid patients already substituted with T_4 to determine the minimal dose required to achieve a normal S TSH level Similarly an adequate T_4 dose was established in previously untreated hypothyroid patients by measuring S TSH In patients given T_4 for suppressive purposes we examined the TSH response to TRH at different dose levels of T_4 The present paper reports our experience of monitoring T_4 doses by means of S TSH determinations and TRH test

STUDY BASE

The study population comprised 55 patients (45 women and 10 men mean age 57) receiving T_4 (Levaxin® Nyegaard Oslo Norway) for primary hypothyroidism and 57 patients (47 women and 10 men mean age 48) receiving T_4 for suppressive purposes

The diagnosis of primary hypothyroidism was invariably confirmed by clinical symptoms and laboratory findings including low S-T_4 or protein bound iodine in combination with a low T_3 resin uptake value indicating low saturation of the thyroid hormone binding proteins Seventeen of the hypothyroid patients had a history of radioiodine treatment and 10 of surgical treatment of thyrotoxicosis None of these patients had a palpable goitre Thirty five hypothyroid patients had been substituted with 0 10–0 25 mg T_4 for 1–5 years Irrespective of their previous dose levels all these patients were given a substitution dose of 0 10 mg T_4 for five weeks This dose was then increased stepwise by 0 05 mg every five weeks until the S TSH was suppressed to below 8 mU/l The remaining 20 hypothyroid patients had not been previously treated They were given an initial dose of 0 05 mg T_4 and here too the T_4 dose was increased by 0 05 mg every five weeks until the S-TSH was suppressed to levels within the normal range S-TSH S-T_4 S-T_3 and T_3-resin uptake were determined after each 5-week interval This interval was chosen because the time necessary for the hypothalamic pituitary thyroid axis to become stabilized has been estimated to be 35 days following alterations in the administration of exogenous thyroid hormone (12)

The indication for suppressive thyroxine therapy was either a goitre (17 cases) or previous subtotal resection of an atoxic goitre (40 cases) No clinical or laboratory signs of hypothyroidism were present when thyroxine therapy began The patients had received 0 10–0 20 mg T_4 for times varying between 5 months and 4 years The T_4 dose was 0 10 mg in 20 patients 0 15 mg in 17 and 0 20 mg in 20 The dose had been unchanged for at least 5 weeks prior the TRH test

METHODS

S-TSH was measured by radioimmunoassay using a commercial kit (Phadebas TSH test Pharmacia Uppsala Sweden) On the basis of studies on normal subjects the discriminatory level between normal and thyroid subfunction states was set at 8 mU/l Patients were given 0 20 mg TRH (TRF (Roche) Hoffman-LaRoche Diagnostica, Basel Switzerland) i v at about 8 a m Venous blood samples were drawn at 0 20 and 60 min for the determination of S-TSH

S-T_4 was determined by column chromatography using an automated method for the final reaction (18) The normal range was 57–145 nmol/l S T_3 was determined by radioimmunoassay (T_3-RIA The Radiochemical Centre Amersham UK) The normal range was 1 2–2 9 nmol/l T_3 resin uptake test was performed using Sephadex as adsorbent The method was a modification of that described by Hansen (7) The normal range was 80–120% of the resin uptake of pooled human serum

RESULTS

Replacement thyroxine dose

In most cases the dose necessary to suppress S TSH to values within the normal range was 0 10–0 15 mg T_4 and no patient in this series needed more than 0 20 mg T_4 (Table I) The dose necessary to normalize S TSH invariably abolished all symptoms of hypothyroidism

There was no statistically significant correlation between the T_4 dose needed and age or body weight The mean S-T_4 and S T_3 as well as the mean T_3-resin uptake fell to values within the normal range at the final T_4 dose level (Table II) There was a statistically significant difference ($p<0.05$) between the T_3 resin uptake values at the higher (0 15–0 20 mg) and the lower (0 05–0 10 mg) dose levels but corresponding differences with regard to S T_4 and S T_3 were not statistically significant

Table I Dose of l thyroxine needed to suppress TSH to levels below 8 mU/l in 55 hypothyroid patients

No of pats	T_4 dose (mg)
3	0 05
25	0 10
23	0 15
4	0 20

Table II *Serum concentrations of T_4 and T_3 and T_3-resin uptake values (mean ± S E M) for patients needing different doses of T_4 to normalize S TSH*

T_4 dose (mg)	T_4 (nmol/l)	T_3 (nmol/l)	T_3-resin uptake (%)
0 05–0 10 (78 pats)	114±7	2 0±0 1	89±3
0 15–0 20 (77 pats)	126±8	2 0±0 1	103±5
Normal range	57–145	1 2–2 9	80–120

Suppressive l thyroxine therapy

As shown in Table III a T_4 dose of 0 10 mg permitted a mean rise in S-TSH of about 2 mU/l 20 min after TRH stimulation This rise was statistically significant ($p<0$ 01) In patients suppressed with 0 15 or 0 20 mg T_4 the mean rise in S TSH after TRH stimulation was not statistically significant Ten out of 19 patients on 0 10 mg 3 out of 17 on 0 15 mg and none of the 21 patients on 0 20 mg T_4 displayed a rise in S TSH of more than 2 mU/l after TRH stimulation The mean S T_4 concentration was above the normal range at a dose of 0 20 mg T_4 but this was not seen at doses of 0 10 and 0 15 mg (Table III) The mean S T_3 and the mean T_3 resin uptake values were within the normal range at all dose levels examined

DISCUSSION

The present results confirm those presented by previous authors (5 19) with regard to the adequate replacement dose of T_4 The essential finding from this and the above investigations is that a sufficient replacement dose need seldom exceed 0 20 mg T_4 If a higher dose is required the patient may not be taking his T_4 tablets regularly or some abnormality may exist in his T_4 absorption metabolism or in the peripheral effect of T_4 Inborn errors of metabolism are known to occur in many metabolic pathways and it would not be surprising if they also exist in T_4 metabolism Indeed failure to respond at all to peroral T_4 administration has been reported (14)

It was previously assumed that maintenance of euthyroidism by T_4 substitution was entirely dependent on this hormone thereby necessitating a supranormal level of S T_4 compared with normal persons in whom euthyroidism is maintained by both T_4 and T_3 Recent findings (3 17 20) concerning the extrathyroidal conversion of T_4 to T_3 indicate that this view is incorrect and it is obvious that the calorigenic effect of T_4 is to a great extent mediated by deiodination of T_4 to T_3 This explains why supranormal S T_4 levels are not needed to attain normal S TSH levels

The determination of S-TSH can today be made on a routine basis and it therefore seems advisable to use it in addition to clinical evaluation in order to obtain a suitable replacement dose of T_4 thereby avoiding unnecessarily high doses of T_4 during life long substitution The possible but as yet unproved disadvantages of taking an unnecessarily high T_4 replacement dose may be speculated upon It is known for instance that overt thyrotoxicosis may be a casual factor in osteoporosis and it is possible that decades of overtreatment with thyroid hormones may detrimentally influence skeletal metabolism It is also possible that cardiac arrhythmias are in some way dependent on the serum level of thyroid hormones There probably exist twilight zones between euthyroidism and manifest hyper and hypothyroidism and various symptoms

Table III *Serum concentrations of TSH during TRH test T_4 and T_3 and T_3-resin uptake values (mean ± S E M) at various dose levels of suppressive T_4 medication*

T_4 dose (mg)	TSH during TRH test (mU/l) 0 min	20 min	60 min	T_4 (nmol/l)	T_3 (nmol/l)	T_3-resin uptake (%)
0 10 (19 pats)	2 6±0 4	4 6±0 6	3 8±0 4	126±9	2 0±0 2	91±4
0 15 (17 pats)	2 4±0 3	2 7±0 4	2 9±0 4	146±6	2 1±0 1	101±2
0 20 (21 pats)	2 1±0 2	2 1±0 2	2 6±0 2	177±8	2 2±0 1	105±2
Normal range				57–145	1 2–2 9	

may occasionally respond to adjustment of the thyroid hormone level in these zones This may be exemplified by the well known fact that angina pectoris in myxoedematous patients may be avoided by keeping the patient in a slightly hypothyroid state

The diminishing effect of thyroxine on atoxic goitre has been stressed by many authors (1 13) using different types of thyroid hormone regimens The value of long term thyroxine suppressive therapy using daily doses of 0 20–0 30 mg in order to prevent the reappearance of goitre after subtotal resection of atoxic goitre has been demonstrated (2) In this context it should be observed that the old concept of increased TSH secretion secondary to iodine deficiency or enzymatic deficiencies in hormone production as a main factor in goitrogenesis is under reevaluation in view of recent findings Thus most investigators report no elevation in S TSH in goitre patients (8 15 21) with the exception of patients from highly endemic areas (11) However even if TSH does not play any role in goitrogenesis a reduction of the TSH production rate to subphysiological levels by means of exogenous thyroid hormone may be of importance in diminishing goitre size and preventing goitre formation

The present report provides some clues to the T_4 dose relevant in this context T_4 in a dose of 0 10 mg has obviously only a minor effect on the pituitary TSH release induced by TRH while 0 20 mg T_4 avoids any significant TRH induced release of TSH in most cases A standard suppressive dose should therefore probably not be lower than 0 20 mg This dose also diminished radioiodine thyroid uptake to 10% of the normal value (16) In suppressive treatment of vital importance for instance after thyroidectomy due to thyroid carcinomas an increase in the T_4 dose up to a level just below clinical hyperthyroidism is clearly advisable

As previously mentioned S TSH alone or in combination with TRH stimulation seems to be the most adequate laboratory analysis for clinical evaluation of thyroxine therapy The analysis of S T_4 S T_3 or T_3 resin test seldom adds any information of practical value S T_3 may in fact be misleading because it is subject to many extrathyroidal influences thus it may be abnormally low without clinical hypothyroidism in elderly patients and in patients with non thyroidal illness (4)

REFERENCES

1 Astwood E B Cassidy C E & Aurbach G P JAMA 174 459 1960
2 Bergfelt G & Risholm L Acta Chir Scand 126 531 1963
3 Braverman L E Ingbar S H & Sterling K J Clin Invest 49 855 1970
4 Chopra I J Chopra V Smith S R Reza M J & Solomon D H J Clin Endocrinol Metab 41 1034 1975
5 Evered D Young E T Ormstone B J Menzies R Smith P A & Hall R Br Med J 3 131 1973
6 Hall R Amos J Garry R & Baxton R L Br Med J 2 274 1970
7 Hansen H Ugeskr Læger 126 1471 1964
8 Hershman J M & Pittman J A Ann Intern Med 74 481 1971
9 Kahn A Can Med Assoc J 109 279 1973
10 Karlberg B Linköping University Medical Dissertation No 8 1973
11 Kochupillai M Deo M G Karmarkas M G McKendrick M Weightman N Evered D C Hall R & Ramalingaswami V Lancet I 1021 1973
12 Krugman L G Hershman J H Chopra I J Levine G A Pekary A E Geffner D L & Chua Teco G N J Clin Endocrinol 41 70 1975
13 Lamberg B A Hernberg C A & Hakkila R Acta Endocrinol 25 1196 1965
14 Ljunggren J G & Persson B Acta Med Scand 197 471 1975
15 Mayberry W E Charib H Bilstad J M & Sizemore C W Ann Intern Med 74 471 1971
16 Oddie T H Fisher D A & Eppersen D J Clin Endocrinol 25 1196 1965
17 Pittman C S Chambers J B & Read W H J Clin Invest 50 1187 1971
18 Schleusener H Acta Endocrinol (Kbh) (Suppl) 184 121 1974
19 Stock J M Surks M I & Oppenheimer J H N Engl J Med 290 529 1974
20 Surks M I Schadlow A R Stock J M & Oppenheimer J H J Clin Invest 52 805 1973
21 Utiger R D J Clin Invest 44 1277 1965

Acta Med Scand 202 261–264 1977

Idiopathic Hypoparathyroidism and Hyperthyroidism

An Exceptional Combination

Per G Farup

From the Department of Medicine Gjøvik Fylkessykehus Gjøvik Norway

ABSTRACT A case of idiopathic hypoparathyroidism is reported The patient had presented symptoms for 16 years and various treatments and diagnoses had been tried before the correct conclusion was reached Adequate treatment resulted in normalization of most of the symptoms The patient also had hyperthyroidism, an exceptional combination A possible relationship between the two diseases is discussed

Idiopathic hypoparathyroidism (IDH) is a rare disease more frequent in women than in men (10) It may occur at any age mean age of onset is around 20 years (6 10 20) Due to insidious onset and lack of characteristic symptoms years may pass before the correct diagnosis is established (10 20) The cause of the illness is unknown but an autoimmune process has been suggested as a possibility and the disease often occurs in patients presenting antibodies against different organs or a latent or manifest organic insufficiency most often Addison s disease pernicious anemia hypothyroidism hypogonadism or diabetes mellitus (4 6 16) Below will be reported a case of idiopathic hypoparathyroidism associated with hyperthyroidism which according to the literature is an extremely unusual combination To the author s knowledge only two cases have been reported (9 24)

CASE REPORT

The patient is a woman born in 1913 Reportedly her first attacks of convulsions occurred in 1956 and were slight and accompanied by general indisposition pains in the extremities and carpal spasms no loss of consciousness She had been under considerable physical and mental stress From 1958 she was given 0 1 g Phenemal three times a day which was reduced to 0 1 g once a day from 1959 In 1961 she had a grand mal seizure and was admitted to a neurologic department where hypocalcemia was demonstrated (serum calcium 2 9 m Eq/l) As tetany was suspected she was referred to our department On admission serum calcium was 3 5 mEq/l and phosphorus 6 2 mg/100 ml Daily calciferol injections were given for five days in addition calcium tablets During hospitalization she felt quite comfortable and had no epileptiform seizures The cause of the hypocalcemia was not determined A follow up was arranged but the patient never turned up

She had had bilateral cataract in 1963 and 1964 and was operated on on both occasions In 1970 and 1972 she was treated for moniliasis Her goiter had been markedly enlarged for years From 1973 a mental change set in characterized by restlessness increasing dementia hallucinations recurrent seizures tremor stridorous dyspnea and sweating Doxepin and promazine chloride had no beneficial effect on her mental symptoms apparently rather the opposite

On readmission in Aug 1974 the patient was uneasy restless shivering and seemed markedly demented BP was 180/85 Chvostek and Trousseau s signs were positive tendon reflexes normal The skin was thin fine warm but not moist There was marked finger tremor Due to the cataract operations eye changes were present modest exophthalmosis The goiter was diffusely enlarged firm without nodules and a slight bruit was audible

Laboratory findings Hb was 12 6 g/100 ml serum creatinine 1 0 mg/100 ml blood urea 67 mg/100 ml creatinine clearance 55 ml/min fasting blood sugar 88 mg/100 ml Oral glucose tolerance test showed a slightly diabetic trend Blood gases and serum natrium kalium and chlorides were normal Initial serum calcium was 2 4–3 2 mEq/l serum phosphorus 5 3–6 2 mg/100 ml and serum magnesium 1 3–1 5 mEq/l Hypocalciuria (less than 1 mEq/24 h) hypophosphaturia (0 2 g/24 h) and hypomagnesiuria (1 7 mEq/24 h) were present Serum total protein was 65 g/l serum albumin 24 g/l The urine was normal pH 6 Oral xylose and vitamin A absorption tests were normal Fecal fat content was normal Neither anti

19 Stamp T B C Effects of long term anticonvulsant therapy on calcium and vitamin D metabolism Proc R Soc Med 67 64 1974
20 Steinberg H & Waldron B R Idiopathic hypoparathyroidism An analysis of fifty two cases including the report of a new case Medicine 31 133 1952
21 Wacker W E C & Parisi A F Magnesium metabolism N Engl J Med 278 712 1968
22 Williams G T & Brown M Laryngospasm in hypoparathyroidism J Laryngol Otol 88 369 1974
23 Wuttke H & Kessler F J Magnesiumkonzentration im Serum bei Hyperthyreose Med Klin 71 235 1976
24 deYeaza M M & Stinebaugh B J Idiopathic hypoparathyroidism with hyperthyroidism South Med J 65 246 1972

Acta Med Scand 202 265–269 1977

Total and Subtotal Colonoscopy with Short Instrument and without Fluoroscopy

Jean Cronstedt Sven Ingmar Deurell and Peter Vestergaard

From the Department of Medicine Bollnas Hospital Bollnas Sweden

ABSTRACT The technique and results of 111 consecutive colonoscopies with a short colonoscope without the use of X ray screening are reviewed The caecum could be reached in 44% of the cases No complications occurred The great clinical value of the procedure is shown by the following findings X ray negative lesions—including 2 cases of carcinoma—were found in 35% of the cases radiologically demonstrated lesions could be defined more precisely in 18%, and the presence of colonic lesions could be ruled out in 11% in spite of equivocal X ray findings It is concluded that many more centres ought to take up the procedure, which offers the prospect of significantly reducing suffering and death from colonic disease

The recent sweeping developments in fibre-optic endoscopy represent the greatest advance in clinical gastroenterology since the advent of diagnostic radiology S C Truelove 1976

Flexible fibre optic colonoscopes were developed in the early 1960s but problems related to the anatomy and contents of the colon created considerable difficulty for early investigators (5 11 12) Over the past 6 years improvements in instrument design and techniques of insertion have led to a number of reports describing the clinical value of colonoscopy (7 15 17) and several reviews have been published (2 13 19) Yet the need for meticulous bowel preparation the technical difficulties and time involved in the procedure itself the initial expense of the instruments and the need for X ray facilities have discouraged many physicians from taking up colonoscopy

This article reviews the results of 111 consecutive colonoscopies performed in the Endoscopy Unit of the Department of Medicine Bollnas Hospital with a short colonoscope and without the use of X ray screening from the time of introduction of the method in March 1975 to the end of 1976

METHODS

All the colonoscopies in this series were performed with the Olympus CF MB2 the only instrument available in the hospital at the time This instrument is 112 cm long and has one biopsy channel only The Olympus CLE 3 cold light supply was used All examinations were performed in the special endoscopy room of the Endoscopy Unit by the same endoscopist who at the beginning of the period concerned had a 7 year experience of upper gastrointestinal endoscopy but only limited experience of colonoscopy

The patients were admitted to the hospital on the morning prior to the day of colonoscopy were given 30 ml of castor oil on admission and were put on a liquid diet In the evening a tap-water washout was given On the following morning two tap-water washouts were given and clear fluids only were allowed for breakfast Thereafter alternating saline and tap-water enemas were given until returns were clear

The patients were given 500 mg of paracetamol (Alvedon® Draco) orally one hour before the colonoscopy Anticholinergics were not given since they can cause atony of the bowel and make intubation difficult Immediately before the examination 5–10 mg of diazepam (Stesolid MR Dumex) were given i v after special care had been taken to explain the procedure to the patient realizing that a few well chosen words before any endoscopic procedure are worth many mg of diazepam The anal region was anesthetized with 5% lignocaine ointment (Xylokain salva 5% Astra)

The patients were examined in the supine position Before insertion of the instrument the controls and light source were checked A few drops of peppermint oil were added to the suction bottle to stop odours Neither a stiffening tube nor X ray screening were used

One endoscopy assistant inserted and withdrew the instrument and a second assistant manipulated the instrument through the abdominal wall by applying pressure

Table I *Indications for colonoscopy*

Colonic symptoms not explained by barium studies	46
Assessment of known or suspected colitis	46
Abnormal or equivocally abnormal areas demonstrated on barium enema	13
Assessment of colon in cases of known terminal ileitis	3
Control after previous surgery for colonic cancer	3
	111

in various directions according to instructions given by the examiner Special attention was paid to the position of the illuminated tip of the instrument which could often be seen through the abdominal wall Seeing the tip was particularly helpful when the junction between the sigmoid and the descending colon was negotiated and when the instrument began to emerge from the splenic flexure The examiner was free to use both hands to work the buttons and controls of the instrument The assistant inserting the instrument frequently viewed down a "teaching aid" which was used during the rest of the examination by a second doctor for true teaching purposes At all times an attempt was made to pass the tip down the centre of the lumen keeping to the "inside curve" whenever possible Minimal air insufflation was used and when progress was difficult the instrument was pulled back and reinserted with different torque By careful steering often using continuous insertion-withdrawal the sigmoid colon could often be made to concertina itself over the instrument and the descending colon was reached directly When the mucosa stopped moving by and whitened or when the patient complained of pain the instrument was immediately withdrawn a short distance in order to avoid the risk of bowel perforation

The sigmoid and its junction with the descending colon re undoubtedly the most difficult areas to negotiate Initially it was occasionally necessary to perform an "alpha loop manoeuvre in order to reach the descending colon but the need for this manoeuvre decreased with experience On reaching the splenic flexure by deflecting and hooking the tip withdrawal of the instrument usually straightened the sigmoid sufficiently Insertion into the transverse colon was greatly facilitated by the second assistant holding the sigmoid firmly from the outside preventing sigmoid loops from reforming The transverse colon was recognized by its triangular form When a redundant loop of the transverse colon was formed it could be straightened out by hooking the tip and withdrawing thus again telescoping the colon over the instrument The hepatic flexure was negotiated by hooking the tip down and again withdrawing Straightening of the transverse colon was often associated with advancement of the tip into the ascending colon Further passage into the caecum was facilitated by suction of air and by the assistant holding the transverse colon upwards

Lesions were noted and occasionally biopsied during insertion but the most careful examination was performed during the withdrawal Photographs and biopsies were taken and the distance from the anus was noted in each instance The biopsies were examined by Professor A Bergstrand and Professor J Eriksson Patodiagnostic Laboratory Stockholm

The time required for the colonoscopy varied between 20 and 80 min the tendency being for shorter time with increasing experience At the end of the procedure the instrument and its channels were washed carefully and sterilized with 2 5% glutaraldehyde solution As a rule the patients were discharged on the morning after the examination except in cases requiring further in patient treatment for their bowel disease

PATIENTS

Since our unit was the only one in the district of Gavleborg performing colonoscopies at the time patients were also referred for examination from three other hospitals in the district—Gavle Hudiksvall and Söderhamn Altogether 111 colonoscopies were performed in 108 patients Their ages varied between 19 and 81 49 being below 40 and 9 above 70 years of age

The indications for colonoscopy are shown in Table I

All patients except one had had a barium enema prior to colonoscopy The exception was a patient with a uretero-sigmoidostomy considered to be a relative contraindication to such barium studies

RESULTS

Technical results and complications

In general an attempt was made to reach the caecum The effort spent on overcoming technical difficulties encountered during the advancement of the instrument varied considerably however according to the assessed potential value of going beyond a certain point In cases of ulcerative proctitis for instance the finding of an entirely normal sigmoid and descending colon *obviously made further* advancement rather unnecessary The caecum was *reached in 46 patients Excluding* 6 cases with organic stricture of the sigmoid there were 11 pa

Table II *Diagnostic results of colonoscopy*

One patient had no barium enema because of specific contraindication

Normal findings in patients with normal barium enema		41
		19
Definition of a radiological lesion		39
Lesions found in X ray negative cases		
X ray negative carcinoma	2	
X ray negative polyps (single or multiple)	19	
X ray negative colitis	18	
Negative findings in equivocal X ray findings		11
		110

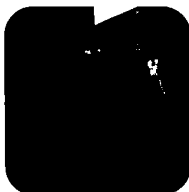


Fig 1



Fig 2



Fig 3

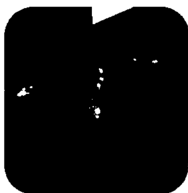


Fig 4

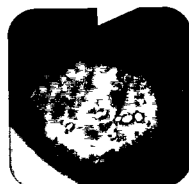


Fig 5



Fig 6

Fig 1 S vere Crohn colitis not seen on barium enema Transverse colon *Fig 2* Ulcerative colitis as seen in the sigmoid *Fig 3* Ulcerative colitis with inflamma tory polyps in descending colon *Fig 4* Caecum normal app arance *Fig 5* Adenomatous sigmoid polyp with severe atypia *Fig 6* Early carcinoma in splenic flexure not seen on barium enema

tients in whom the sigmoid only could be examined because of sigmoid loops forming despite attempts made to prevent this In the remaining 48 patients the examination included the descending colon in 20 half of the transverse colon in 13 and the whole transverse colon in 15

In all patients preparation of the colon had been very efficient Consequently there was no need in any case to limit the examination due to residual colonic contents

Only one patient experienced considerable pain during the procedure He had had an abdominal operation only a few weeks earlier and his case emphasized the importance of not performing colonoscopy too soon after abdominal surgery The other patients experienced very little pain during the examination and reported it as instructed when they did Such pain ceased immediately when the instrument had been withdrawn a short distance No serious complications such as perforations prolonged bleeding after biopsy or myocardial infarctions occurred

Diagnostic results

The diagnostic results of the 111 colonoscopies are shown in Table II

In 19 patients radiologically demonstrated lesions were defined more precisely In 3 cases of sigmoid stricture endoscopy revealed carcinoma in 2 and segmental Crohn s disease of the colon in the third Radiological diagnosis of colitis was endoscopically and histologically confirmed in 13 cases Diverticulosis only was found in one case of rectal bleeding In one case terminal ileitis was endoscopically and histologically confirmed no signs of colitis having been found This patient was the only one in this series in whom the terminal ileum could be entered with the instrument In one patient a suspected caecal polyp was shown to be a postoperative appendiceal invagination

Definite lesions were discovered in 39 X ray negative cases Carcinoma was found in 2 of these patients One of them had already metastasized at operation whereas the other was an early cancer X ray negative polyps of varying size—two of them more than 3 cm long—were demonstrated in 19 patients Single or multiple polyps were found in the sigmoid in 13 cases in the descending colon in 4 in the transverse colon in 2 and in the ascending colon in 2 cases The majority of the polyps were sessile pedunculated ones being demonstrated in only 4 patients Since endoscopic polypectomy was not introduced until the end of 1976 definite histological classification of the polyps was not obtained in all cases Five patients underwent operation showing superficial carcinoma in 1 villous adenoma in 2 inflammatory polyps in 1 and lymphangioma in 1 In the other 14 patients the histological classification of the polyps was based on biopsies alone showing villous adenoma in 1 adenomatous polyps in 7 metaplastic polyps in 2 and inflammatory polyps in 2

X ray negative colitis was found in 18 patients Three of them (19–22 years of age) had Crohn s disease of the colon causing a sigmoid stricture in one and ulcerations of various sizes separated by areas of normal mucosa in the other two Subtotal ulcerative colitis of moderate to severe degree was demonstrated in 8 patients Two patients had mild total colitis and 5 mild subtotal colitis The endoscopic findings in these patients covered the whole spectrum from minimal change inflammatory bowel disease with vascular changes only to severe colitis with thick mucopurulent exudate associated with ulcerations Biopsy findings confirmed the endoscopic diagnosis of colitis in all these cases but the type of colitis was difficult to determine in the small biopsy specimens Non specific or chronic colitis of varying degrees was reported in 13 cases ulcerative colitis in 3 and Crohn s disease of the colon in 2

Colonoscopy was negative in 11 patients with equivocal X ray findings In 8 of them the radiological suspicion of colitis could not be endoscopically verified No stricture could be found in three cases of suspected sigmoid stricture one of whom had marked spastic contractions in the colon

DISCUSSION

It has been estimated that adenomatous polyps occur in up to 20% of colons (1 8) and that 4% of the population may have a polyp of 1 cm or more in diameter (18) Evidence is mounting that adenomatous polyps may become malignant (9) and it seems likely that most carcinomas do go through a polypoid stage at least early in their development (18) Additional lesions can be demonstrated in as many as 40–50% of patients going to surgery for a single lesion seen on X ray (4) and carcinomas of varying degrees of invasiveness have been found in as many as 20% of these patients (20)

15 Waye J D Colonoscopy Surg Clin North Am 52 1013 1972
16 — Colonoscopy without fluoroscopy Gastrointest Endosc 21 198 1975
17 Williams C B & Muto T Examination of the whole colon with the fibreoptic colonoscope Br Med J 3 278 1972
18 Williams C B & Riddell R H Colonic polyps and polypectomy In Modern topics in gastrointestinal endoscopy (ed K F R Schiller & P R Salmon) p 253 Heinemann Medical Books London 1976
19 Williams C B & Teague R Progress report Colonoscopy Gut 14 990 1973
20 Winawer S J Melamed M & Sherlock P Potential of endoscopy biopsy and cytology in diagnosis and management of patients with cancer Clin Gastroenterol 5 575 1976
21 Winawer S J Schottenfeld D Miller D Sherlock P Deschner E Watson R Edelman M Fleischer M Schwartz M Hajdu S & Melamed M Screening for cancer and polyps of the colon Abstract of the Third International Symposium on Detection and Prevention of Cancer 1976

Acta Med Scand 202 271–276 1977

Acute Hepatitis
A Prognostic Study with Observation Time up to 37 Years

A Follow up of the Iversen/Roholm Liver Biopsy Material

O Dietrichson H Zoffmann P Christoffersen M Hilden
E Juhl and Å Chr Thomsen

From Departments of Medicine II and III Kommunehospitalet Department of Medicine B Bispebjerg Hospital the Division of Hepatology and the Department of Pathology Hvidovre Hospital University of Copenhagen Denmark

ABSTRACT Re evaluation of 890 consecutive liver biopsies from 1939–59 gave the diagnosis of acute hepatitis in 147 patients A follow up study of these patients was performed 15–37 years after the diagnostic biopsy, based on repeated liver biopsies, biochemical liver tests autopsy reports and death certificates Two patients died from acute liver failure and development of cirrhosis was documented or strongly suspected in 22 patients (15%) A comparison between these 24 patients with a malignant course of hepatitis and 86 patients with a well documented uncomplicated disease, revealed a significantly larger number of women a higher age, and more cases with piece meal necrosis confluent necrosis and marked portal inflammation in the initial liver biopsy in the group with the poor prognosis

In 1939 Iversen and Roholm (9) presented the first results of a liver biopsy technique We have re evaluated the biopsy specimens which Iversen collected in 1938–59 and in order to shed light on the prognosis of acute hepatitis we have undertaken a follow up study of the patients who displayed the morphological changes of this disease

In addition a comparison has been made between the variables recorded initially in patients with uncomplicated acute hepatitis and in those who subsequently developed chronic liver disease or died from acute liver failure

MATERIAL AND METHODS

The basis for the study is a material of 890 consecutive percutaneous liver biopsies performed in Department of Medicine III Kommunehospitalet Copenhagen during 1938–59 All the biopsy specimens were re evaluated without any knowledge of the clinical diagnosis For technical reasons no histological diagnosis could be established in 97 specimens (11%) Acute hepatitis was diagnosed in 160 biopsies (20% of the remaining material) from 147 patients and it is these patients who are represented in the present study (Fig 1)

The histological diagnosis of acute hepatitis is based on internationally agreed criteria (2) and was made when the following five changes were present provided that the lobular structure was preserved and there was no severe fibrosis in the portal spaces focal liver cell necrosis variation of cells and nuclei Kupffer cell proliferation and acidophilic bodies (13)

During the period May 1974–Feb 1976 (15–37 years after the primary biopsy) a follow up study of the 147 patients was performed based on information obtained from various Public Records Offices in Denmark the Office of Medical Statistics the National Health Service the National Archives the Municipal Archives University of Copenhagen general hospitals general practitioners and individual personal contacts

Sixty five patients were still alive at the time of the follow up study A repeated liver biopsy according to the method of Menghini was performed in 17 of them (16–35 years after the initial biopsy) In a further two patients a follow up biopsy taken 24 and 26 years after the acute disease was available for evaluation These 19 patients and a further 39 were interviewed Biochemical liver tests (aspartate aminotransferase bilirubin alkaline phosphatase and albumin) were carried out and serum was examined for the presence of hepatitis B surface antigen (HB_sAg) by immunoelectroosmophoresis (14) Five of the patients still alive refused to participate one was on the tramp in Denmark and one had emigrated to England

Eighty two patients had died This group was evaluated on the basis of information available such as autopsy (47 cases) and death certificates case records from hospitals and general practitioners (33 cases) Two patients were

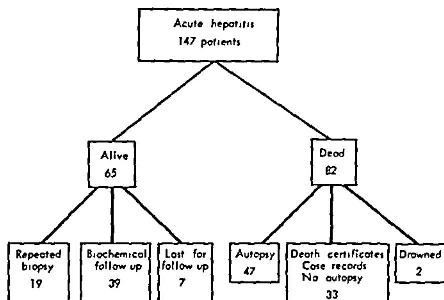


Fig 1 Follow up of 147 patients with acute hepatitis

sailors who had drowned in foreign waters so that no further information was available regarding them

The non parametric Mann Whitney test and the χ^2 test were used in the statistical analysis The quantitative variables are recorded by median and range Confidence limits for the binomial distribution are from Geigy Scientific Tables

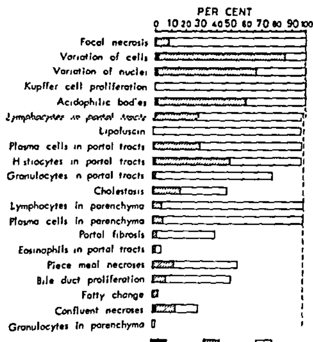


Fig 2 Morphological features in 147 patients with acute hepatitis

RESULTS

Fig 2 shows the morphological findings in the initial biopsies of the 147 patients including the five diagnostic features mentioned as well as the remaining abnormal inconstant histological findings

The age and sex distributions of the patients are shown in Fig 3 The age range was 15–80 years (median 45) and 56% were men There were more women (56%) than men over 50 years of age

As illustrated in Fig 4 the cumulative survival curve for the patients is significantly lower than that of the Danish population in 1950 matched for sex and age

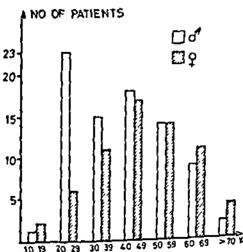


Fig 3 Age and sex distribution of 147 patients with acute hepatitis

Table I *Data on 26 patients with acute or chronic liver disease (CLD) when last examined or at the time of death*

Age at onset (y)	Sex	Year of onset	Years between onset and diagnosis of CLD	Observation period	Alcohol abuse	Autopsy or biopsy diagnosis	Main cause of death
47	♂	1939	–	1 d	?	Acute hepatitis	Biopsy complication
40	♂	1943		6 d	?	Acute hepatitis	Biopsy complication
45	♀	1938		1 d	?	Acute hepatitis	Acute liver failure
44	♂	1939		2 mo	0	Acute hepatitis	Acute liver failure
60	♂	1940	2	4 y	0	Cirrhosis	Liver failure
25	♀	1942	12	32 y	0	Cirrhosis	Liver failure
45	♀	1944	<1	1 y	?	Cirrhosis	Liver failure
70	♀	1944	2	2 y	?	Cirrhosis	Liver failure
57	♀	1944	<½	1 y	0	Cirrhosis	Liver failure
64	♀	1945	10	10 y	0	Cirrhosis	Liver failure
58	♀	1945	<½	6 mo	?	Cirrhosis	Liver failure
57	♀	1945	<½	4 mo	?	Cirrhosis	Liver failure
48	♀	1947	<½	1 mo	?	Cirrhosis	Liver failure
45	♂	1939	19	19 y	0	Cirrhosis	Liver failure
54	♀	1957	1	5 y	0	Cirrhosis	Liver failure
69	♀	1958	1	11 y	0	Cirrhosis	Liver failure
54	♀	1947	22	22 y	0	Cirrhosis	Heart disease
74	♀	1954	4	7 y	?	Cirrhosis	Apoplexia
69	♀	1944	7	7 y	?		Liver failure
50	♀	1944	1	1 y	?		Liver failure
61	♂	1944	<1	8 mo	?		Liver failure
71	♂	1950	<½	5 mo	?		Liver failure
64	♀	1957	1	10 y	?		Liver failure
70	♀	1945	6	6 y	?		Pneumonia
80	♀	1948	1	1 y	?		Heart disease
39[a]	♀	1944	14	24 y	0	Cirrhosis	

[a] Alive

Cirrhosis was suspected in only one of the 19 patients who underwent repeated biopsy. This patient had no history of alcoholic abuse. No pathological changes, fatty liver or non specific changes were found in the biopsy specimens of the others.

The further 39 patients interviewed reported an uncomplicated course of the hepatitis and had no symptoms of liver disease at the time of the follow up. Two of them (one alcoholic) had slightly elevated alkaline phosphatase but normal values for the other biochemical liver tests. None of the patients investigated had circulating HB Ag.

Autopsy was carried out in 47 of the dead patients and revealed liver disease in 18, 14 of whom had died from liver failure. The remaining 29 autopsies disclosed no sequela of hepatitis.

Chronic liver disease was reported in seven of the 33 dead patients without autopsy and according to the death certificates liver failure was the cause of death in five.

Table I gives more detailed information concerning the 26 patients (1 alive and 25 dead) with evidence of liver disease when last investigated or at the time of death. Two died within the first week from severe haemorrhage caused by the biopsy, two from acute liver failure, 17 from liver failure *after* development of chronic liver disease, four

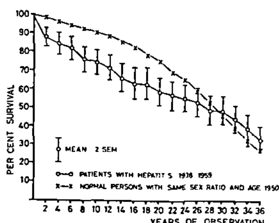


Fig. 4. Survival curves of 147 patients with acute hepatitis and of persons in the Danish population of the same sex and age.

7 Cullinan E R King R C & Rivers J S Br Med J 1 1315 1958
8 Dietrichson O Juhl E Christoffersen P Elling P Faber V Iversen K Nielsen J O Petersen P & Poulsen H Acta Pathol Microbiol Scand (A) 83 183 1975
9 Iversen P & Roholm K Acta Med Scand 102 1 1939
10 Jersild M N Engl J Med 237 8 1947
11 Krarup N B & Roholm K Acta Med Scand 108 306 1941
12 Neefe J R Gambescia J M Kurtz C H Smith H D Beebe G W Jablon S Reinhold J G & Williams S C Ann Intern Med 43 1, 1955
13 Petersen P Christoffersen P Elling P Juhl E Dietrichson O Faber V Iversen K Nielsen J O & Poulsen H Scand J Gastroenterol 9 607 1974
14 Prince A M & Burke K Science 169 593 1970
15 Schaefer J W Schiff L Gall E A & Oikawa Y Am J Med 42 348 1967
16 Sherlock S Lancet 1 817 1948
17 Zieve, L Hill E Nesbitt S & Zieve B Gastroenterology 25 495 1953

Acta Med Scand 202 277–281 1977

Presymptomatic Primary Biliary Cirrhosis

Lad Hanik and Sten Eriksson

From the Department of Internal Medicine University of Lund Malmo General Hospital Malmo Sweden

ABSTRACT Clinical, laboratory and follow up results in 13 patients with primary biliary cirrhosis (PBC) collected during the last 10 years in a well defined population of 250 000 inhabitants are presented The mean observation time was 5 5 years 77% of these patients have been asymptomatic for many years Characteristic laboratory features in asymptomatic patients are high alkaline phosphatases and glutamyl transpeptidases and very high levels of polyclonal IgM ESR is often increased Signs of active cell destruction are slight and functioning cell mass is well preserved High titers of mitochondrial antibodies are consistently present Needle biopsy is seldom sufficient for diagnosis but permits staging of the disease There is no correlation between clinical features and histological evolution stage Compared with a preceding 10 year period, the incidence of PBC has risen threefold This increase can be fully explained by the extended use of laboratory facilities, resulting in the detection of asymptomatic patients

Primary biliary cirrhosis (PBC chronic non suppurative destructive cholangitis) in its classical form presents with itching and jaundice of cholestatic type in middle aged women with patent extrahepatic bile ducts Other less common and often later symptoms include skin pigmentation xanthelasmata and xanthomas and portal hypertension The diagnostic evaluation including biochemical serological and histological methods in these symptomatic patients is usually conclusive Among the chronic liver diseases PBC is rare In a retrospective analysis from this hospital in 1963 including 360 cases of liver cirrhosis diagnosed in a 10-year period only 4 (1 1%) were though to represent PBC (9)

In recent years we have observed an increasing number of asymptomatic patients with PBC In this paper we describe the typical features of these patients Special emphasis is put on diagnostic criteria in asymptomatic patients with PBC their prognosis and the relationship between absence of symptoms and phase of disease

PATIENTS AND METHODS

The study is based on 13 patients diagnosed in 1965–75 and regularly seen in the Department of Medicine Malmo General Hospital The city has 250 000 inhabitants and only one hospital The majority of patients with chronic liver disease are accordingly referred to our department The patients comprise 9 women mean age 57 years (range 42–70) and 4 men mean age 48 5 years (range 41–55) They have all been seen at 3–4 month intervals Mean observation time is 5 5 years (range 2–10) Patent bile ducts have been documented in all patients with standard X ray methods

The *biochemical screening* includes determination of p bilirubin aspartate aminotransferase (ASAT) alanine aminotransferase (ALAT) glutamyltranspeptidase (GT) alkaline phosphatase (ALP) prothrombin and cholesterol according to standard methods Plasma protein analysis has been performed in the Department of Clinical Chemistry using electroimmunoassay (13)

Dynamic liver function tests include bromsulphalein (BSP) elimination determined 30 min after i v injection of 5 mg BSP/kg b wt and demethylation of ^{14}C dimethyl aminopyrine (DMA) as described by Hepner and Vesell (11) with determination of $^{14}CO_2$ in expired air 2 hours after peroral administration of ^{14}C DMA

Immunological investigations include determination of mitochondrial antibodies (AMA) and smooth muscle antibodies (SMA) using the indirect immunofluorescence technique modified after Walker et al (20) and antibodies against cytoplasmic thyroid antigen using the complement binding technique and in some cases immunofluorescence technique

HB_sAg was determined by immunoelectroosmophoresis (10) and rheumatoid factor by sheep red cell agglutination Cytomegalovirus antibodies were determined by complement fixation tests Paul Bunnel's test was used for mononucleosis Thyroid stimulating hormone (TSH) was determined by radioimmunoassay All immunological determinations were performed in the National Bacteriological

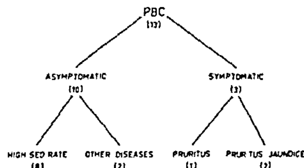


Fig 1 Presenting symptoms in 13 patients with PBC

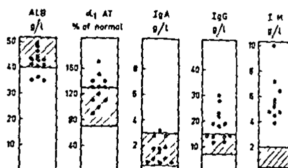


Fig 3 Plasma proteins *ALB*=albumin α_1 *AT*=α_1 antitrypsin Other symbols as in Fig 2

Laboratory Stockholm and the Department of Bacteriology Malmo General Hospital

Histology Needle biopsies in all patients were performed with the Menghini needle and interpreted and staged according to Scheuer s criteria (16) Surgical wedge biopsies have not been performed in any patient as there was no indication for laparotomy

RESULTS

No patients had a family history of liver or collagen disease Signs of rheumatic or thyroid disorders were absent Excess alcohol consumption was noted in one patient No drug history was obtained

As shown in Fig 1 10 patients had no hepatobiliary complaints at the time of diagnosis Three patients had itching and in two of these jaundice also ppeared within 6 months after diagnosis Hepatoegaly was noted in 4 asymptomatic and in all ymptomatic patients The biochemical findings are summarized in Figs 2–4 Only initial values are included Bilirubin was moderately elevated in all symptomatic patients Asymptomatic patients were anicteric ASAT and ALAT levels were slightly increased ALP and GT exhibited more marked elevations The levels tended to be higher in symptomatic than in asymptomatic patients Cholesterol was moderately elevated in less than half of the patients Prothrombin levels were normal in all

Fig 3 gives some plasma protein levels at the initial evaluation The albumin level is normal in most patients α_1 antitrypsin slightly increased in approximately half the patients IgA levels are within normal limits A pronounced polyclonal increase in IgM is the most prominent feature of the plasma protein profile High levels of IgG were found in approximately half the patients Other plasma proteins (orosomucoid haptoglobin fibrinogen) were essentially normal in all patients Ceruloplasmin was slightly elevated in half of the patients

Results of dynamic liver function tests are shown in Fig 4 BSP elimination was abnormal in all but 3 subjects in contrast to the demethylation capacity which was abnormal in only 2 of the 10 patients investigated

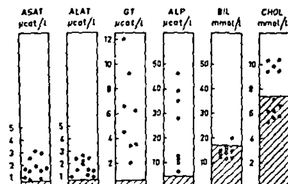


Fig 2 Liver tests *BIL*=bilirubin *CHOL*=cholesterol ●=symptomatic O=asymptomatic patients ▨=normal range

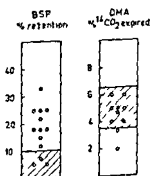


Fig 4 Dynamic liver function tests *BSP*=bromsulphalein *DMA*=^{14}C-dimethylaminopyrine Other symbols as in Fig 2

Table I *Liver histology in relation to clinical stage*

	Asymptomatic	Symptomatic
PBC stage		
I, II	8	
III	1	2
IV	1	1
No of pats	10	3

All patients exhibited significant titers (>1/100) of AMA at repeated examinations SMA were absent in all except one symptomatic patient in whom an increasing titer of SMA was noted during progress of the disease Tests for ANF and rheumatoid factor were negative High titers (often >1/5000) of antibodies against cytoplasmic thyroid antigen were present in all patients (complement fixation) but all were clinically euthyroid with normal TSH levels Investigations for cytomegalovirus infection and mononucleosis were consistently negative All patients were HB_sAg negative

The histological findings in needle liver biopsies when interpreted according to Scheuer (16) were in all cases compatible with the diagnosis of PBC but only in two cases virtually diagnostic The partition of various stages among patients is shown in Table I The histological picture was more advanced (stages III–IV) in symptomatic than in asymptomatic patients but it should be noted that in 2 asymptomatic patients advanced changes were already present at the initial investigation

The progress of the disease is illustrated in Fig 5 During a mean follow up time of 5 5 years slight itching appeared in only two initially asymptomatic patients after 1–2 years The majority of patients remained clinically healthy and the laboratory values were essentially unchanged during these years Signs of active inflammation are slight Of the 3 symptomatic patients only one has shown a rapid progress to deep jaundice Of the patients in this series 4 have undergone immunosuppressive treatment with prednisone and/or azathioprine for 3–6 months In no case could any biochemical or histological improvement be seen

DISCUSSION

The most impressive feature of this small but carefully followed series of patients with PBC is the high incidence (77%) of asymptomatic patients with no hepatobiliary symptoms at all and a silent apparently non progressive course The reason for referral to hospital (Fig 1) has been an unexplained high ESR in the majority of patients sometimes combined with moderate hepatomegaly This has led to investigation of liver enzymes and a plasma protein analysis The findings of high levels of ALP and GT and marked polyclonal increase in IgM and sometimes an isolated increase in α_1 antitrypsin without concomitant increase in other acute phase reactants have directed the diagnostic considerations towards liver disease and PBC Our results favour the concept that this diagnosis should be suspected in the asymptomatic patient with the following constellation of laboratory findings high levels of ALP and GT but only moderate elevations of transaminases high polyclonal IgM and high titer of AMA Patent extrahepatic bile ducts should be demonstrated at this stage by X ray Determination of plasma cholesterol and bilirubin is of no diagnostic aid being normal in most patients Normal bilirubin (Fig 2) is likewise found in 20% of symptomatic patients (17)

The functioning parenchymal cell mass is normal or near normal in most of these patients as judged from normal prothrombin and albumin levels (Fig 3) Further documentation of this fact was obtained by the DMA breath test (Fig 4) considered to be a quantitative liver function test (11) The BSP retention on the other hand is abnormal in most patients but this probably does not reflect a reduced cell mass but rather a reduced number of receptors available for binding Competition with bile acids for binding sites is possible but purely hypothetical However it should be remarked that the highest

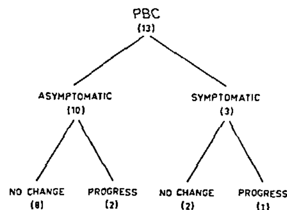


Fig 5 Progress of the disease

BSP retentions occurred in the two asymptomatic patients with subnormal DMA test and the most advanced histological findings

High levels of polyclonal IgM have been reported in 80–100% of patients with PBC (2 6) and are probably a reflection of increased synthesis (6) In this series as in another (1) there is no correlation between IgM level and duration or severity of disease High IgM levels are in no way diagnostic of PBC appearing reversibly in mononucleosis hepatitis A and cytomegalovirus infection (1 21) conditions definitely not present in our patients In those diseases the increase in IgM is as a rule much less impressive Idiopathic high IgM levels are not rare

AMA should be determined in patients with suspected PBC being positive in 84–96% of reported cases of PBC but only in 0 7% of healthy individuals (5 20) AMA were detected in significant titers at repeated examinations of all our patients The antibodies in the present material as in another (12) were found to be associated mainly with IgG and only in two cases with a simultaneous low IgM titer Like IgM AMA level has no relation to severity or duration of disease being just a marker of an autoimmune process and lacking absolute specificity as it is positive in 25% of patients with chronic hepatitis and cryptogenic cirrhosis (4 19)

Autoimmune thyroiditis is sometimes associated with PBC but in the present series all patients were inically euthyroid and furthermore had normal TSH and lacked antibodies against thyroid globulin Thyroid cells known to be rich in mitochondria react with AMA (20) This fact explains the high titers of antibodies against cytoplasmatic thyroid antigen in all our patients investigated with complement fixation tests

The histological diagnosis of PBC or chronic non suppurative destructive cholangitis (15) has been discussed extensively in Scheuer s monograph (16) The appearance of needle biopsy specimens is more often compatible with the condition than diagnostic due to small sample size Diagnostic changes in such specimens are found in only 15–30% compared with 50–70% in surgical wedge biopsies (12 17) In accordance with these findings diagnostic changes in this series were present in only 2 patients the others being compatible The possibility of diagnostic liver biopsy confirmation of PBC is further limited by the strong histological similarity between advanced stages of PBC and chronic active hepatitis (CAH) as illustrated in a study from the Mayo Clinic (8) It is evident that even repeated needle biopsies are of limited value in confirming the diagnosis of PBC

It is well known that immunosuppressive treatment is of limited value in PBC but it has recently been suggested (8) that a therapeutic trial can be used in the differentiation between CAH and PBC Four patients in this series were subjected to such a trial without any evidence of biochemical or histological improvement However it should be noted that a failure to respond to such treatment was observed in 20% of patients with CAH having predominant cholestatic features

The results presented here and by others (7 14) show that PBC can be diagnosed in the asymptomatic patient Suitable screening tests are ALP IgM and AMA Reports on asymptomatic patients with PBC are sporadic In large series published by Sherlock (17) and Klatskin and Kantor (12) comprising several hundred patients with PBC approximately 10% are reported to have been asymptomatic In contrast more than 2/3 in our series are asymptomatic The difference probably reflects selection factors our patient series being representative of a well defined population

The relationship between clinical and histological stage is important Fox et al (7) found only early histological changes in their presymptomatic patients On the other hand PBC patients with bleeding due to portal hypertension as the first manifestation and without histological signs of cirrhosis have been described (22) Among our presymptomatic patients we found one woman with established (stage IV) and another with stage III cirrhosis It is obvious that PBC can progress silently and that lack of symptoms need not correlate with an early histological stage

Follow up studies of asymptomatic patients are rare Klatskin and Kantor (12) mention the appearance of symptoms in only one out of 22 patients but give no data on observation time In a preliminary English series (14) 8 of 21 patients presented symptoms after 2 years follow up Two of our asymptomatic patients have acquired pruritus 1–2 years after diagnosis Although our patients as judged from transaminase and α_1 antitrypsin levels have a low grade inflammatory process we cannot exclude a slow transition into a definite cirrhosis

For several reasons one cannot make a strict comparison of the incidence of PBC during the last

10-year period with the period 1953–63 (9) mentioned earlier but the results do suggest an approximately 3 fold increase The number of symptomatic cases is essentially the same during the two periods It is therefore evident that the increased incidence can be explained by the addition of asymptomatic cases that would not have been detected 20 years ago The extensive use of laboratory facilities i e access to autoanalyzers individual determinations of immunoglobulins and determination of AMA is probably responsible for this development

In view of these data it is conceivable that PBC is more common than previously suspected and that the symptomatic cases perhaps represent only the top of an iceberg (7) We speculate that many asymptomatic patients can very slowly pass into a definite cirrhosis which at autopsy can be classified as cryptogenic due to absence of histological signs typical of PBC

There is no effective therapy for PBC Despite the recently reported trials with penicillamine treatment (3) we do not think its use is justified in asymptomatic patients due to the high frequency of side effects

REFERENCES

1 Bevan G Baldus W P & Gleich G J Serum immunoglobulin levels in cholestasis Gastroenterology 56 1040 1969
2 Brunner G Vido I & Schmidt G Die Diagnose der primar biliaren Zirrhose Dtsch Med Wochenschr 97 1448 1972
3 Deering T B Dickson E R Geall M G Fleming C R McCall J T & Goldstein N P The effect of D-penicillamine on urinary copper excretion in primary biliary cirrhosis Gastroenterology (Abstract) 69 A 16/816 1975
4 Doniach D Walker J G Roitt I M & Berg P A "Autoallergic hepatitis N Engl J Med 282 86 1970
5 Editorial Mitochondrial antibodies in collagen disorders Br Med J 1 460 1974
6 Feizi T Immunoglobulins in chronic liver disease Gut 9 193 1968
7 Fox R A Scheuer P J & Sherlock S Asymptomatic primary biliary cirrhosis Gut 14 444 1973
8 Geubel A P Baggenstoss A H & Summerskill W H J Responses to treatment can differentiate chronic active liver disease with cholangitic features from the primary biliary cirrhosis syndrome Gastroenterology 71 444 1976
9 Hallén J & Krook H Follow up studies on an unselected ten year material of 360 patients with liver cirrhosis in one community Acta Med Scand 173 479 1963
10 Hansson B G & Johnsson T Improved technique for detecting Australia antigen by immunoelectroosmophoresis Vox Sang 21 531 1971
11 Hepner G W & Vesell E S Quantitative assessment of hepatic function by breath analysis after oral administration of ^{14}C aminopyrine Ann Intern Med 83 632 1975
12 Klatskin G & Kantor F S Mitochondrial antibody in primary biliary cirrhosis and other diseases Ann Intern Med 77 533 1972
13 Laurell C B Electroimmuno assay Scand J Clin Lab Invest (Suppl) 124 21 1972
14 Long R G Scheuer P J & Sherlock S Asymptomatic primary biliary cirrhosis Gut (Abstract) 17 390 1976
15 Rubin E Schaffner F & Popper H Primary biliary cirrhosis Am J Pathol 46 387 1965
16 Scheuer P J Liver biopsy interpretation pp 33–39 Baillière Tindall London 1973
17 Sherlock S The presentation and diagnosis of 100 patients with primary biliary cirrhosis In The liver and its diseases pp 227–235 International Medical Books Co New York 1974
18 Sipponen P Orcein positive hepatocellular material in long standing biliary diseases Scand J Gastroenterol 11 545 1976
19 Walker J G Doniach D & Doniach I Mitochondrial antibodies and subclinical liver disease Q J Med 39 31 1970
20 Walker J G Doniach D Roitt I M & Sherlock S Serological tests in diagnosis of primary biliary cirrhosis Lancet 1 827 1965
21 Wollheim F & Williams R C Studies on the macroglobulins of human serum I Polyclonal immunoglobulin class (IgM) increase in infectious mononucleosis N Engl J Med 274 61 1966
22 Zeegen R Stansfeld A G Dawson A M & Hunt A H Bleeding oesophageal varices as the presenting feature in primary biliary cirrhosis Lancet 2 9 1969

Acta Med Scand 202 283–287 1977

Serum Bile Acids in Man during Vitamin C Supplementation and Restriction

Anders Kallner

From the Department of Clinical Chemistry Karolinska Institutet at Huddinge Hospital Stockholm Sweden

ABSTRACT A daily dosage of 5 g ascorbate was given to 14 persons during one month Various routine biochemical parameters were studied and the concentrations of individual bile acids in serum were determined A significant increase in chenodeoxy cholic acid concentration was found on interruption of vitamin C supplementation, whereas no other changes in bile acid concentrations were significant One person used to a large ascorbate intake (1 g/day) was deprived of ascorbate On resuming the high ascorbate intake, serum bile acid concentrations showed an increasing trend Urinary oxalate excre tion and concentrations were impressively increased during vitamin C supplementation but no effects on kidney function were observed

Bile acids are produced in the liver by oxidation of cholesterol The bile acids are distributed between two main pools the liver and the intestine including the gallbladder In a recent review Krumdieck and Butterworth (14) summarized several investigations dealing with the effect of ascorbate on cholesterol metabolism It was shown in a recent study (2) that when guinea pigs were given a vitamin C free diet the activity of the 7α hydroxylating system of liver microsomes was decreased A similar approach to investigate the effect of vitamin C on cholesterol metabolism in man is virtually impossible

In the present report two experimental models were used In one the daily dosage of ascorbate was increased far above the normal and changes in the bile acid pattern in serum were followed during one month In the other one subject was on a large daily dosage for three months and the daily intake was then drastically reduced

SUBJECTS

Vitamin C 5 g (1 25 g×4 C vimin® Astra Sodertalje) was given to 14 male volunteers below 25 years of age for 4 weeks To the best of our knowledge they were healthy and all laboratory test values were within normal limits No restrictions in diet were applied One subject was given 1 g/day of vitamin C for 3 months and the supplementation was then discontinued During a period of one week vitamin C rich food was avoided and the supplementation was then resumed Samples were taken from all subjects after a night s fasting Samples from vitamin C supplemented subjects were investigated before supplementation and at two and four weeks After interruption of supplementation samples were taken on days 1 2 5 and 9 The samples are numbered I II III IV V and VI respectively Samples were taken daily from the other test persons under identical premises

METHODS

Chemical methods

Triglycerides cholesterol calcium creatinine and aminotransferases were determined by routine methods in the Department of Clinical Chemistry Phosphate was de termined by a malachite-green method (12) Ascorbate in serum and urine was determined as described by Gyorgy (8) This method determines the serum concentration of ascorbate and dehydroascorbate Oxalate in urine was determined by a commercial laboratory (17) by a titri metric method after precipitation

Bile acids were determined by gas–liquid chromatography after isolation and concentration on XAD-2 (Serva) according to the method of Sjovall and Masuri (19) Recovery of bile acids was calculated by addition of a tracer amount of 24-^{14}C tauro-cholate (Radiochemical Center Amersham) to the serum sample before dilution and chromatography Recoveries were be tween 92 and 97% Lithocholic acid was used as internal standard in gas-chromatographic analysis added before derivation of bile acids The areas under the peaks were measured by planimetry and the calculated was corrected with regard to recovery data in each

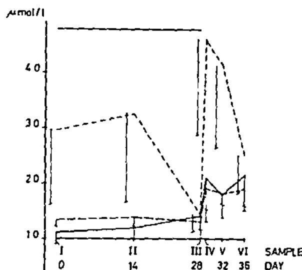


Fig 1 Concentrations of serum bile acids during vitamin C supplementation —=cholic acid =deoxycholic acid =chenodeoxycholic acid Vertical bars to the left of the value for chenodeoxycholic acid=S E M Horizontal bar=period of vitamin C supplementation

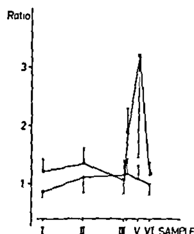


Fig 2 Ratios C/D (Δ—Δ) and CD/D (□—□) during vitamin C supplementation Abbreviations as in Table II

Statistical methods

t Values were calculated using Student s *t* test for correlated data

RESULTS

Routinely determined serum and urine parameters before and during administration of vitamin C are shown in Table I After two weeks of supplementation no values showed increases except those directly dependent on the compound administered (ascorbate and oxalate) Decreases were found in creatinine and phosphate Phosphate rose again during the second half of the test period to the same level as before the supplementation Creatinine increased to a level significantly above the initial level but still within normal limits

The bile acid concentrations in serum in the group supplemented with ascorbate are given in Table II and Figs 1 and 2 A significant increase judged by the sign test (20) was found for chenodeoxycholate between the last day of vitamin C supplementation and the first day without (samples III and IV) A return to previous values was obtained during the following week The ratios of cholic acid and chenodeoxycholic acid to deoxycholic acid were calculated but no significant changes were recorded during the period of observation

Serum bile acid concentrations in the deprivation test with only one subject are shown in Fig 3 All

Table I *Serum and urine concentrations before (I) after 2 (II) and 4 (III) weeks of vitamin C supplementation (mean±S E M)*

Differences are significant at the 95% level for $t \geq 2$ 16

	Reference value	I	II	III	*t* I/II	*t* II/III	*t* I/III
S-cholesterol (mmol/l)	<7 8	4 83±0 23	4 78±0 23	4 99±0 23	0 64	−1 51	−1 29
S triglycerides (mmol/l)	<1 7	0 9±0 08	0 9±0 07	0 9±0 08	0 63	−0 15	0 35
S-creatinine (µmol/l)	<120	88±2	79±2	97±3	3 68	−4 25	−2 74
S-calcium (mmol/l)	2 20–2 70	2 53±0 02	2 51±0 0	2 54±0 0	0 20	−0 22	−0 08
S phosphate (mmol/l)	0 7–1 6	1 3±0 04	1 2±0 03	1 3±0 02	2 97	−3 12	0 70
S ASAT (µkat/l)	<0 70	0 27±0 01	0 29±0 02	0 26±0 02	−1 26	2 07	0 54
S ALAT (µkat/l)	<0 70	0 27±0 03	0 33±0 05	0 26±0 03	−1 56	2 16	0 27
S ascorbate (µmol/l)	30–85	79±3	130±6	102±6	−8 92	3 27	−3.54
U ascorbate (µmol/24 h)	–	942	11 695	13 115			
U-creatinine (mmol/24 h)	7–19	19±4	19±4	20±4	0 43	−1 76	−1 04
U-oxalate (µmol/24 h)	–	49±16	1 295±744	1 540±601	−6 21	−1 12	−9 22

Table II *Serum bile acid concentrations (μmol/l) at different times during vitamin C supplementation (mean±S E M)*

Sample no	Cholic acid (C)	Deoxycholic acid (D)	Chenodeoxycholic acid (CD)	C/D	CD/D
I	1 11±0 208	1 34±0 123	2 96±1 350	0 87±0 11	1 22±0 20
II	1 19±0 220	1 38±0 166	3 25±1.592	1 11±0 26	1 35±0 26
III	1 40±0 392	1 31±0 153	1 43±0 318	1 15±0 25	1 07±0 22
IV	2 10±0 808	1 91±0 382	4 59±1 732	1 17±0 26	1 88±0 42
V	1 79±0 416	1 81±0 204	4 13±1 503	1 08±0 23	3 20±1 75
VI	2 15±0 649	1 89±0 306	2 50±0 688	1 00±0 19	1 17±0 18

Table III *Serum bile acid concentrations (μmol/l) during vitamin C restriction in one subject*

Sample no	Cholic acid (C)	Deoxycholic acid (D)	Chenodeoxycholic acid (CD)	C/D	CD/D
1	0 60	3 57	5 09	0 18	1 43
2	0 76	1 35	1 27	0 58	0 94
3	1 32	1 86	2 22	0 74	1 19
4	1 37	1 90	1 45	1 10	1 12
5	0 81	1 30	1 94	0 65	0 88
6	0 93	1 35	1 32	0 72	0 98
7	0 73	1 40	0 97	0 55	0 69
8	1 08	1 22	0 97	0 92	0 79
9	1 38	1 81	2 32	0 80	1 28
10	1 91	1 30	1 50	1 53	1 16

bile acids decreased to new levels which were not essentially changed until supplementation was resumed Concentrations and ratios are shown in Table III

DISCUSSION

Spittle (21) observed that vitamin C supplementation lowered serum cholesterol to various degrees in different age groups and proposed that this could be explained by an increased metabolism of cholesterol and a mobilization of cholesterol deposits Ginter et al (6) have shown that the higher the initial cholesterolemia the greater the hypocholesterolemic effect after a prolonged administration of ascorbic acid Also Davies and Newson (4) have shown a positive correlation between serum cholesterol and both plasma and leucocyte ascorbate In a short term experiment (6 weeks) Fix et al (5) found no significant change in the cholesterol levels

The rate limiting step in the conversion of cholesterol to bile acids the 7α hydroxylation is specifically reduced in ascorbate-deficient guinea pigs (2) On the other hand the activity of HMG

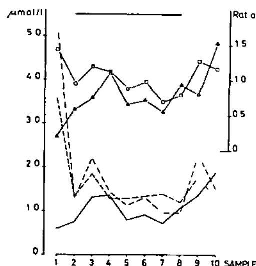


Fig 3 Concentrations of serum bile acids and ascorbate in one subject during vitamin C restriction Horizontal bar=period of vitamin C restriction Symbols as in Figs 1 and 2

coenzyme A reductase which catalyzes the rate limiting step in the cholesterol biosynthesis was also decreased in these animals (3) The content of cholesterol in the liver of these animals was increased which could be interpreted as illustrating the total effect of both rate limiting steps There was no change in the serum levels of cholesterol

The level of serum bile acids might be a sensitive indication of liver function (13) and it has been suggested that the liver is capable of removing a constant fraction of bile acids in the enterohepatic circulation (16)

Quantitative or qualitative changes in the bile acid synthesis might be reflected in the bile acid pattern of serum A significant increase of short duration in chenodeoxycholate concentration was found in the present study after discontinuation of vitamin C supplementation The individual changes are great and minor trends may be obscured by the biological variations

The formation of gallstones in hamsters can be decreased by high doses of ascorbate (7) This could rather be a general effect of the cholesterol bile acid ratio than of an endogenous increase in chenodeoxycholic acid synthesis The effect of ascorbic acid on biliary lipid composition in man is not significant but Pedersen (18) only determined the total bile acid content in bile not the individual bile acids

There is evidence that dihydroxy bile acids and in particular chenodeoxycholic acid are absorbed from the intestine at a higher rate than cholic acid (6) It has been reported recently (11) that ascorbate lowers the absorption of bile acids from the intestine in guinea pigs in particular the absorption of chenodeoxycholic acid This is in agreement with the findings in the present study that a decrease in the ascorbate intake causes an increase in the serum chenodeoxycholate level On the other hand the above authors obtained a large increase in the biliary output of chenodeoxycholic acid on intraperitoneal administration of ascorbate

Only one of the present subjects was studied after a sudden reduction of the ascorbate intake from a comparatively high daily dosage to almost nought Such a rapid large change in dosage might possibly enhance the effects of a lowered dosage and simulate a stage of relative deficiency A decrease in the dihydroxy bile acid concentration in serum was indicated when the high ascorbate intake was discontinued

One of the metabolites of ascorbic acid in man is oxalate and with the dose given in this study, relatively large amounts would be expected in the urine (15 22) On a molar basis about 5% of the ascorbate given was recovered in the urine After administration of 1 ^{14}C ascorbate about 2% of the isotope has been recovered in the oxalate fraction of urine (9) It is unlikely that a high oxalate concentration per se would form precipitates (1)

ACKNOWLEDGEMENTS

This study was supported by grants from Karolinska Institutet Stockholm and Astra AB Södertälje Sweden

REFERENCES

1 Backman L, Bergström K & Hallberg D Kidney stone—a complication caused by intestinal shunt in treatment of obesity Läkartidningen 72 462 1975
2 Björkhem I & Kallner A Hepatic 7α hydroxylation of cholesterol in ascorbate-deficient and ascorbate supplemented guinea pigs J Lipid Res 17 360 1972
3 Björkhem I & Kallner A The activity of HGM coenzyme A reductase in ascorbate-deficient and ascorbate supplemented guinea pigs To be published
4 Davies J O G & Newson J Ascorbic acid and cholesterol levels in pastoral people in Kenya Am J Clin Nutr 27 1039 1974
5 Fix J Davies J A & Copenhaver J H Vitamin C and serum cholesterol levels in human Nebr Med J 59 (9) 324 1974
6 Ginter E Cerna O Budlovsky J Balaz V Hruba F Roch V & Sasko E Effect of ascorbic acid on plasma cholesterol in humans in a long term experiment Int J Vitam Nutr Res In press 1977
7 Ginter E & Mikus L Reduction of gallstone formation by ascorbic acid in hamsters Experentia In press 1977
8 György P Vitamin methods Vol 1 p 303 New York 1950
9 Hammarström L Autoradiographic studies on the distribution of C^{14} labelled ascorbic acid and dehydroascorbic acid Acta Physiol Scand (Suppl) 289 47 1966
10 Hislop I Hofmann A F & Schoenfield L Determinants of the rate and site of bile acid absorption in man J Clin Invest 46 1070 1967
11 Iwamoto K Ozawa N Ito F Okamoto N & Watonabe J Effect of ascorbic acid on the intestinal absorption of bile salts and metabolism of cholesterol in guinea pigs Chem Pharm Bull 24 (9) 2014 1976
12 Kallner A Determination of phosphate in serum and urine by a single step malachite green method Clin Chim Acta 59 35 1975
13 Kaplowitz N Kok E & Javitt N B Postprandial serum bile acid for the detection of hepatobiliary disease JAMA 225 292 1973

14 Krumdieck C & Butterworth C E Ascorbate–cholesterol–lecithin interactions factors of potential importance in the pathogenesis of atherosclerosis Am J Clin Nutr 27 866 1974
15 Lamden M P & Chrystowski G A Urinary oxalate excretion by man following ascorbic acid ingestion Proc Soc Exp Biol Med 85 190 1954
16 La Russo N F Korman M G Hoffman N E & Hofmann A F Dynamics of the enterohepatic circulation of bile acids Postprandial serum concentrations of conjugates of cholic acid in health cholecystectomized patients and patients with bile acid malabsorption N Engl J Med 291 689 1974
17 Medicinsk Laboratorium Copenhagen Denmark (Hodgkinson A & Williams A) Clin Chim Acta 36 127 1972
18 Pedersen L Biliary lipids during vitamin C feeding in healthy persons Scand J Gastroenterol 10 311 1975
19 Sjovall J & Masuri T A versatile method for analysis of bile acids in plasma Anal Lett 5 341 1972
20 Snedecor G W & Cochran W G Statistical methods 6th ed pp 127 and 554 Iowa State University Press Ames Iowa 1967
21 Spittle C R Atherosclerosis and vitamin C Lancet 2 1280 1971
22 Takenouchi K Aso K Kawase K Ideikowa H & Shiomi T On the metabolites of ascorbic acid especially oxalic acid eliminated in urine following the administration of large amounts of ascorbic acid J Vitaminol 12 49 1966

Acta Med Scand 202 289–293 1977

The Liver in Sarcoidosis

Eero Lehmuskallio Matti Hannuksela and Helena Halme

From the Department of Dermatology University Central Hospital Helsinki Finland

ABSTRACT Needle biopsies of the liver were performed in 121 cases of sarcoidosis Granulomas compatible with sarcoidosis vere seen in 24% of the cases Liver function tests (serum alkaline phosphatase, serum aspartate aminotransferase serum alanine aminotransferase, and bromsulphthalein clearance test) were performed on 325 patients with sarcoidosis and on 132 with non sarcoid erythema nodosum (EN) Pathological findings were seen especially in patients with extensive EN, without any correlation with the disease responsible for the eruption Hepatic granulomas were found more often in patients with sarcoid changes in lung parenchyma than in those with bilateral hilar adenitis only There were no other definite correlations between hepatic granulomas and other clinical and laboratory findings The incidence of pathological results in this study was clearly lower than, e g , in the USA, thus reflecting the good prognosis of sarcoidosis in the Scandinavian countries

Liver biopsy and liver function tests are widely used in studying hepatobiliary disorders in sarcoidosis Sarcoid granulomas in the liver are found in about 2/3 of the cases in autopsy materials (7) and in 71% using peritoneoscopy and liver biopsy (29) On the other hand serum alkaline phosphatase (S-AP) and serum aspartate aminotransferase (S ASAT) are seldom elevated in uncomplicated sarcoidosis (26) but often elevated in sarcoid erythema nodosum (EN) (12) The bromsulphthalein clearance test (BSP) has been found to be abnormal in severe cases of the disease (27) Portal hypertension jaundice and other severe disturbances of liver function are rare and most of the cases reported have been North American coloured patients (25)

This paper reports the results of our studies on liver function in Finnish sarcoid patients with a good prognosis

PATIENTS AND METHODS

The series consisted of 325 patients with untreated sarcoidosis examined at the Department of Dermatology University Central Hospital Helsinki The diagnosis was considered to be confirmed in 287 of the patients for whom in addition to consistent clinical features the Kveim test was positive and/or biopsy evidence of sarcoidosis was obtained from lymph nodes skin bronchial mucosa or spleen In the remaining 38 cases the diagnosis was based only on the findings in a chest X ray and on the natural course of the disease Some clinical data of the patients are given in Table I

Because of the large number of patients with EN in the sarcoidosis group 132 patients with non sarcoid EN were collected as control series Their clinical data too are given in Table I

The laboratory tests for liver function were S AP S ASAT serum alanine aminotransferase (S ALAT) and BSP The normal ranges for S ASAT and S ALAT (17) were 0 08–0 33 μkat/l 25 C In BSP bromsulphthalein 7 5 mg/kg b wt was injected intravenously According to the method of Tovey (30) blood samples were taken at 0 5 15 25 35 50 and 60 min Drawn on semilogarithmic paper the values formed a biphasic regressive line The first phase represents the saturation of the reticuloendothelial system and the second the excretion of the dye from the liver *K* values (excretion rate) over 0 020 were considered to be normal those between 0 010 and 0 020 indeterminate and those below 0 010 distinctly pathological S AP was determined according to Bessey et al (5) with normal values 0 8–2 9 BL units or 0 22–0 80 μkat/l 37°C

A liver biopsy was performed using a Manchi puncture needle diameter 1 2 mm Pethidin chloride (50–75 mg) was given intramuscularly 30 min prior to the biopsy The aspiration was performed in deep expirium with a Rekord syringe of 20 ml through the intercostal route The specimen a 5–20 mm long strip of liver was fixed immediately in formaline The stain was hematoxylin-eosin

The microscopic examination was made independently by two investigators Granulomas consisting of 10 or more epithelioid cells were regarded as compatible with sarcoidosis Accumulations of less than 10 epithelioid cells were considered to be non specific alterations The initial interpretation of the two investigators differed in only 4 out of 121 cases and even in these cases agreement was achieved

Table I Clinical data on 325 patients with sarcoidosis and 132 with non sarcoid erythema nodosum (EN)

	Sarcoidosis						
	Subacute			Total		Non sarcoid EN	
	+EN	−EN	Chronic	No	%	No	%
Males	19	51	21	91	28	5	4
Females	119	74	41	234	72	127	96
Age (y)							
−15			2	2	1	2	2
16–25	11	22	1	34	10	30	23
26–35	58	41	4	103	32	46	35
36–45	31	26	25	82	25	28	21
46–55	29	23	16	68	21	11	8
56–65	7	11	12	30	9	12	9
66–	2	2	2	6	2	3	2
Skin sarcoidosis							
Scar	17	12	5	34			
Papular	2	7	8	17			
Nodular		3	13	16			
Ocular sarcoidosis							
Iritis	5	14	11	30			
Chest X ray[a]							
0	7	12	13	32	10		
I	95	59	19	173	53		
II	16	13	6	35	11		
III IV	20	40	21	81	25		
V		1	3	4	1		
Mantoux test[b]							
+to 0 01						10	8
+to 0 1	11	14	12	37	11	31	23
+to 1	50	27	14	91	28	46	35
+to 10	37	41	14	92	28	28	21
+to 100	20	23	7	50	15	7	5
−to 100	19	20	15	54	17	10	8

[a] 0=no changes I=bilateral hilar adenitis (BHA) II=BHA+perihilar infiltrations III=parenchymal changes±BHA IV=miliary mottling±BHA V=fibrosis

[b] TU of PPD RT 23 from Statens Seruminstitutt Copenhagen Denmark

RESULTS

The liver was enlarged (palpable more than 1 cm below the costal margin) in 11% of the sarcoid patients (Table II) Subacute and chronic sarcoidosis did not differ in this respect Patients in a healed or regressive stage of the disease showed liver enlargement more seldom (5%) than those in an active (14%) or static stage (10%)

S ALAT was markedly increased in 3 out of 61 cases examined all of these patients had EN S ASAT was distinctly high in only one out of 267 patients EN was present in this case too

High S-AP values were encountered in 1 3% (4/310) of those examined (Table II) Two of them had subacute sarcoidosis with EN one without but one patient had a chronic form of the disease All 4 of these patients had active sarcoidosis

The BSP excretion rate was distinctly abnormal in 8% of those examined Patients with EN showed pathological values more often than others

The results of the liver function tests mentioned above did not differ significantly from those found in non sarcoid EN (Table II)

Granulomas compatible with sarcoidosis were found in 24% out of 121 specimens obtained from sarcoid patients (Table III) Neither the enlargement of the liver nor the presence of skin sarcoidosis showed a correlation to the presence of granulomas On the other hand hepatic granulomas were seen more often in patients with abnormal

Table II *Size of the liver and the values of S AP S ALAT S ASAT and BSP in patients with sarcoidosis and with non sarcoid erythema nodosum (EN)*

	Sarcoidosis							
	Subacute +EN		Subacute −EN		Chronic		Non sarcoid EN	
	No	%	No	%	No	%	No	%
Size of the liver								
Examined	137		128		60		132	
Enlarged	17	12	11	9	9	15	13	10
S AP (μkat/l 37°C)								
Examined	128		124		58		125	
–0 80	119	93	119	96	51	88	117	94
0 82–1 33	7	5	4	3	6	10	4	3
1 35–	2	2	1	1	1	2	4	3
S ALAT (μkat/l 25 C)								
Examined	31		16		14		31	
–0 33	21	68	11	69	9	64	28	90
0 35–1 00	7	23	5	31	5	36	2	6
1 02–	3	9					1	3
S ASAT (μkat/l 25 C)								
Examined	109		109		49		111	
–0 33	96	88	102	94	42	86	103	93
0 35–1 00	12	11	7	6	7	14	7	6
1 02–	1	1					1	1
BSP (*k*)								
Examined	34		18		11		37	
> 020	23	68	15	83	9	82	32	87
010– 020	8	24	1	6	2	18	4	11
< 010	3	9	2	11			1	3

results of liver function tests than in those with normal values There was also a positive correla tion between sarcoid changes in lung parenchyma and sarcoid involvement in the liver

Neither clinical jaundice nor portal hypertension were found Slightly elevated bilirubin values in the serum which also were within the normal limits a few weeks later were encountered in only two cases

DISCUSSION

S ASAT S ALAT and S AP were distinctly pa thological in a very few cases in this study The results are in accordance with those obtained by Selroos (26) in Finnish sarcoid patients The abnormal values were seen mostly in cases of EN Some years ago the pathological values had been found to reflect the severity of EN but not the causative agent (12)

The bromsulphthalein retention test has been widely used for detecting the dysfunction of liver cells Small doses of bromsulphthalein (2–5 mg/kg b wt) apparently measure only the capacity of the reticuloendothelial system to capture the dye (30) With 5 mg/kg abnormal results have been shown in 33% 44% and 54% of the cases reported by others (14 19 23) A bromsulphthalein dose of 7 5 mg/kg is high enough to permit the determination of changes in the excretion rate By this method we found distinctly abnormal results in 8% of sarcoid cases and indeterminate results in a further 17 5% Pathological values were seen more often in sarcoidosis with EN than in other forms of sarcoidosis

It is very difficult to determine the size of the liver Scadding (25) regarded a palpability of at least one finger s breadth below the costal margin as indicating hepatomegaly The liver was enlarged in only 4 (1 5%) of his 275 patients The correspond ing percentage in Gilg s work (10) was only 0 5% but generally the figures presented have been much higher e g 25% (20) 43% (22) and 55% (16) The liver extended 1 cm or more below the costal mar gin in 11 4% of our patients The great differences

Table III Correlations between the findings in liver biopsy specimens and the results of liver function tests in 121 patients with sarcoidosis

BHA = bilateral hilar adenopathy

	No of pats		
		Granulomas in liver	
	Total	Present	Absent
Liver			
Normal	102	25	77
Enlarged	19	4	15
S AP (μkat/l 37°C)			
Not done	1		1
–0 80	107	24	83
0 82–1 33	12	5	7
1 35–	1		1
S ASAT (μkat/l 25 C)			
Not done	1		1
–0 33	111	25	86
0 35–1 00	9	4	5
1 02–			
BSP (k)			
Not done	95	22	73
> 020	22	4	18
010–020	3	3	0
< 010	1		1
Chest X ray			
Normal	11	5	6
BHA only	64	10	54
Lung changes	46	14	32
Skin sarcoidosis			
Present	28	5	23
Absent	93	24	69

between the percentages presented are apparently due to both the different diagnostic criteria and the differences in severity of the disease between series

Hepatic epithelioid cell granulomas in patients with unexplained fever may present a real diagnostic problem At least tuberculosis (8 9) Hodgkin s disease (1) and sarcoidosis (15) must be looked for Guckian and Perry (11) listed altogether 35 diseases in which granulomas indistinguishable from each other can be found Caseating necrosis is sometimes present in tuberculosis tularemia and brucellosis In addition the causative microorganism can be seen under microscope or found in culture

In sarcoidosis epithelioid cell granulomas have been detected in about 1/2–2/3 of puncture biopsy specimens (1 4 6 14 16 18 19 21 23 28) A more accurate method of detecting granulomas is to take biopsies through peritoneoscopy By this means Tachibana et al (29) found an incidence of about 70% in a Japanese series The number of positive findings in autopsy materials has been on the same level e g 18 of 22 cases (24) 14 of 27 (20) and 14 of 24 (3) The number of puncture biopsies revealing sarcoidosis was only 24% in the present study The great difference between this figure and those reported by other investigators can hardly be due to different methods of taking liver biopsies The natural course of sarcoidosis is clearly more benign in Finland and the other Scandinavian countries than elsewhere (13) This fact explains the discrepancy and also another low figure of positive biopsy rate (20%) found in Scandinavia (14)

A normal finding in a chest X ray does not exclude the possibility of sarcoidosis in patients with hepatic granulomatosis (15) The same was true in the present study in which 5 out of 29 patients with sarcoid involvement in the liver showed no abnormalities in a chest X ray In such cases a positive histological finding from another organ or a positive Kveim test is necessary in establishing the diagnosis

The present finding that patients with bilateral hilar adenopathy without parenchymal infiltration showed hepatic granulomas less frequently than patients with parenchymal changes is easy to explain as a sign of more severe disease Its diagnostic value is however very questionable because non caseating granulomas are seen in 10–25% of cases of lung tuberculosis (19 21)

Portal hypertension and severe liver failure are rare in sarcoidosis (25) No such cases were seen among our patients with good prognosis

REFERENCES

1 Baccharach T Sarcoidosis A clinical review of 111 cases Am Rev Respir Dis 84 12 1961
2 Bagley C M Jr Roth J A Thomas L B & Devita V T Jr Liver biopsy in Hodgkin s disease Ann Intern Med 76 219 1972
3 Barrie H J & Bogoch A The natural history of the sarcoid granuloma Am J Pathol 27 451 1951
4 Behrend H Les formes extra pulmonaires de la sarcoidose La sarcoidose particulièrement dans ses localisations extrathoraciques pp 87–96 Hallwag Genève 1972
5 Bessey O A Lowry O H & Brock M J A method for the rapid determination of alkaline phosphatase with five cubic milliliters of serum J Biol Chem 164 321 1946

6 Bigot R Pariente R Étienne J P Delavierre Ph Chretien J & Brouet G Étude histologique clinique et laparoscopique du foie dans 28 cas de sarcoidoses ganglio-pulmonaires La sarcoidose Rapp de la IV^e Conf Int 1966 (ed J Turiaf & J Chabot) pp 687–697 Masson & Cie Paris 1967
7 Branson J H & Park J H Sarcoidosis—hepatic involvement Ann Intern Med 40 111 1954
8 Dvořáčková I Nerad V & Skaunic V The differential diagnostic problems of tuberculosis and sarcoidosis in granulomatous hepatitis Acta Hepatogastroenterol 20 193 1973
9 Fitzgerald M X Fitzgerald O & Towers R P Granulomatous hepatitis of obscure aetiology Diagnostic contribution of Kveim testing and antituberculous therapy Q J Med 40 371 1971
10 Gilg I Kliniske undersøgelser over Boecks sarcoid (sarcoidose) Behandling och forlob Thesis Cristreus Bogtrykkeri København 1955
11 Guckian J C & Perry J E Granulomatous hepatitis of unknown etiology—an etiologic and functional evaluation Am J Med 44 207 1968
12 Hannuksela M Erythema nodosum with special reference to sarcoidosis Ann Clin Res (Suppl) 7 1971
13 Hannuksela M Salo O P & Mustakallio K K The prognosis of acute untreated sarcoidosis Ann Clin Res 2 57 1970
14 Ingestad R & Tryding N Serum proteins and liver function in sarcoidosis Acta Med Scand 180 449 1966
15 Israel H L & Goldstein R A Hepatic granulomatosis and sarcoidosis Ann Intern Med 79 669 1973
16 Israel H L & Sones M Selection of biopsy procedures for sarcoidosis diagnosis Arch Intern Med 113 255 1964
17 Karmen A Wróblewski F & LaDue J S Transaminase activity in human blood J Clin Invest 34 126 1955
18 Klatskin G & Yesner R Hepatic manifestations of sarcoidosis and other granulomatous diseases—a study based on histological examination of tissue obtained by needle biopsy of the liver Yale J Biol Med 23 207 1950
19 Lebacq E La sarcoidose de Besnier Boeck Schaumann Éditions Arscia Bruxelles 1964
20 Longcope W T & Freiman D G A study of sarcoidosis Medicine (Baltimore) 31 1 1952
21 Mather G Dawson J & Hoyle C Liver biopsy in sarcoidosis Q J Med 24 331 1955
22 Mayock R L Bertrand P Morrison C E & Scott J H Manifestations of sarcoidosis Analysis of 145 patients with a review of nine series selected from the literature Am J Med 35 67 1963
23 Nolan J P & Klatskin G The fever of sarcoidosis Ann Intern Med 61 455 1964
24 Ricker W & Clark M Sarcoidosis A clinicopathologic review of three hundred cases including twenty two autopsies Am J Clin Pathol 19 725 1949
25 Scadding J G Sarcoidosis Eyre & Spottiswoode London 1967
26 Selroos O The frequency clinical picture and prognosis of pulmonary sarcoidosis in Finland Acta Med Scand (Suppl) 503 1969
27 Shay H Berk J E Sones M Aegerter E E Weston J K & Adams A B The liver in sarcoidosis Gastroenterology 19 441 1951
28 Swierenga J Enkele beschouwingen betreffende diagnostiek en therapie van de ziekte van Besnier Boeck Schaumann naar aanleiding van ongeveer 200 geobserveerde patienten tussen de jaren 1950 en 1960 Ned Tijdschr Geneeskd 106 1166 1962
29 Tachibana T Aratake K Okada S Matsuda M Kato S Naito M & Akiyama Y Clinical course of patients with sarcoidosis with hepatic lesions Proc VI Int Conf Sarc 1972 (ed K Iwai & Y Hosoda) pp 382–385 University Park Press Tokyo 1974
30 Tovey J E Bromsulphalein clearance test of liver function Clin Chim Acta 15 149 1967

Table III *Correlations between the findings in liver biopsy specimens and the results of liver function tests in 121 patients with sarcoidosis*
BHA = bilateral hilar adenopathy

	No of pats		
		Granulomas in liver	
	Total	Present	Absent
Liver			
Normal	102	25	77
Enlarged	19	4	15
S AP (μkat/l 37°C)			
Not done	1		1
-0 80	107	24	83
0 82-1 33	12	5	7
1 35-	1		1
S ASAT (μkat/l 25 C)			
Not done	1		1
-0 33	111	25	86
0 35-1 00	9	4	5
1 02-			
BSP (%)			
Not done	95	22	73
> 020	22	*4*	*18*
010-020	3	3	0
< 010	1		1
Chest X ray			
Normal	11	5	6
BHA only	64	10	54
Lung changes	46	14	32
Skin sarcoidosis			
Present	28	5	23
Absent	93	24	69

between the percentages presented are apparently due to both the different diagnostic criteria and the differences in severity of the disease between series

Hepatic epithelioid cell granulomas in patients with unexplained fever may present a real diagnostic problem At least tuberculosis (8 9) Hodgkin s disease (1) and sarcoidosis (15) must be looked for Guckian and Perry (11) listed altogether 35 diseases in which granulomas indistinguishable from each other can be found Caseating necrosis is sometimes present in tuberculosis tularemia and brucellosis In addition, the causative microorganism can be seen under microscope or found in culture

In sarcoidosis epithelioid cell granulomas have been detected in about 1/2–2/3 of puncture biopsy specimens (1 4 6 14 16 18, 19 21 23 28) A more accurate method of detecting granulomas is to take biopsies through peritoneoscopy By this means Tachibana et al (29) found an incidence of about 70% in a Japanese series The number of positive findings in autopsy materials has been on the same level e g 18 of 22 cases (24) 14 of 27 (20) and 14 of 24 (3) The number of puncture biopsies revealing sarcoidosis was only 24% in the present study The great difference between this figure and those reported by other investigators can hardly be due to different methods of taking liver biopsies The natural course of sarcoidosis is clearly more benign in Finland and the other Scandinavian countries than elsewhere (13) This fact explains the discrepancy and also another low figure of positive biopsy rate (20%) found in Scandinavia (14)

A normal finding in a chest X ray does not exclude the possibility of sarcoidosis in patients with hepatic granulomatosis (15) The same was true in the present study in which 5 out of 29 patients with sarcoid involvement in the liver showed no abnormalities in a chest X ray In such cases a positive histological finding from another organ or a positive Kveim test is necessary in establishing the diagnosis

The present finding that patients with bilateral hilar adenopathy without parenchymal infiltration showed hepatic granulomas less frequently than patients with parenchymal changes is easy to explain as a sign of more severe disease Its diagnostic value is however very questionable because non caseating granulomas are seen in 10–25% of cases of lung tuberculosis (19 21)

Portal hypertension and severe liver failure are rare in sarcoidosis (25) No such cases were seen among our patients with good prognosis

REFERENCES

1 Baccharach T Sarcoidosis A clinical review of 111 cases Am Rev Respir Dis 84 12 1961
2 Bagley C M Jr Roth J A Thomas L B & Devita V T Jr Liver biopsy in Hodgkin s disease Ann Intern Med 76 219 1972
3 Barrie H J & Bogoch A The natural history of the sarcoid granuloma Am J Pathol 27 451 1951
4 Behrend H Les formes extra pulmonaires de la sarcoidose La sarcoidose particulièrement dans ses localisations extrathoraciques pp 87–96 Hallwag Genève 1972
5 Bessey O A Lowry O H & Brock M J A method for the rapid determination of alkaline phosphatase with five cubic milliliters of serum J Biol Chem 164 321 1946

Acta Med Scand 202 295–300 1977

Low-Dose Insulin Treatment of Diabetic Ketoacidosis

Peter Claes Eskildsen and Jørn Nerup

From Medical Department F Gentofte/Herlev Hospital Herlev and Steno Memorial Hospital Copenhagen Denmark

ABSTRACT Twenty four consecutively admitted episodes of acute diabetic dysregulation in 22 patients were treated with a low dose insulin regimen, given as hourly i m injections of 5 IU insulin The fall in blood glucose was almost linear during the first 8 hours of treatment, on an average 10% per hour of the initial value The hyperglycemia and acidosis were corrected by 2–12 hours of treatment The deficiency of water and electrolytes, especially potassium, was treated with infusion from the beginning and the fluid balance was corrected within 12–16 hours A severe fall in plasma potassium was never seen, but hypokalemia (<3 6 mEq/l) was still present in some cases after 24 hours of treatment One patient died on account of a large myocardial infarction, but otherwise the patients were restored to habitual condition in 1–4 days The regimen was found to be simple, safe and effective in all cases without risk of late hypoglycemia or severe hypokalemia The study indicates, however, that the parenteral supply of potassium advocated previously 12 5 mEq/hour, is not sufficient when the plasma potassium on admission is below 5 0 mEq/l In such cases it is recommended that the rate of potassium infusion is increased

The application of a low-dose insulin regimen in the treatment of diabetic ketoacidosis (2) has resulted in much controversy concerning the dose and route of administration of insulin in this condition

Needing a simple effective and safe protocol for the treatment of diabetic ketoacidosis in the emergency ward of a university clinic serving a large community we preferred hourly i m injections of small doses of insulin As the effectiveness of this regimen has been discussed and questioned (9 14 15 20 21) we found it reasonable to report our experience In addition we would like to suggest that the rate of potassium infusion originally advocated by Alberti et al (2) is not sufficient in many instances

Reprint requests to P C Eskildsen Medical Department F Herlev Hospital DK 2730 Herlev Denmark

PATIENTS AND METHODS

Twenty four consecutive episodes of diabetic ketoacidosis in 22 patients were treated from Aug 1974 to March 1976 with the low-dose insulin regimen Clinical details of the patients on admission are summarized in Table I Diabetes had not been diagnosed previously in 9 patients in 5 cases the precipitating factor was unknown while clinical signs of infection were present in 13 patients 2 had myocardial infarction and one subarachnoidal bleeding The patients were characterized by marked negative fluid balance depressed level of consciousness and increased respiratory rate but only two were severely hypotensive (BP≤100 mmHg)

The biochemical features of the patients on admission are shown in Table II Twenty one patients were ketoacidotic and 3 non-ketotic but only one of them had hyperosmolar coma (plasma osmolality 353 mosmol/l) Blood glucose (2 8–4 4 mmol/l) sodium (136–147 mEq/l) potassium (3 6–4 9 mEq/l) total carbon dioxide (23–31 mmol/l) creatinine (62–133 µmol/l) protein (57–82 g/l) and Hb (7 3–10 9 mmol/l) were determined by routine methods in the Department of Clinical Chemistry Plasma osmolality was calculated from the molar concentrations of glucose sodium and potassium according to the equation plasma osmolality=2×(Na +K⁺)+gluc =282–308 mosmol/l Results are expressed as mean±S D

Treatment

Following the principles outlined by Alberti et al (2) and described in a previous study (6) (the 11 cases in the latter study are included in the present) the treatment was started as soon as the diagnosis had been established 1) 1000 ml isotonic sodium chloride infused during the first hour followed by 1000 ml every 2nd–4th hour until the blood glucose was below 14 mmol/l then continued infusion with isotonic glucose-containing solutions 2) Insulin Neutral Leo R I ® 10 IU i v and 10 IU i m injected immediately and after 90 min followed by 5 IU i m every hour until the acidosis had disappeared Thereafter the patients were maintained on small dosages of Insulin

Table I *Clinical condition of the patients on admission*

No of episodes	24	
Sex and age	14 ♀ 10 ♂	20–90 y mean 59 15–61 y mean 41
Duration of diabetes and previous treatment	9 4< 11>	not previously diagnosed *10 y all insulin* 10 y 10 insulin 1 diet only
Precipitating factor	5 6 3 2 1 1 1 1 1 2 1	unknown gastrointestinal infection acute pyelitis *undefined viral infection* infected foot gangrene infectious hepatitis insufficient liver function acute pancreatitis dietary break acute myocardial infarction subarachnoidal hemorrhage
Hypotensive (syst BP≤100 mmHg)	2	
Tachycardia (*pulse≥100/min*)	*13*	
Fever (temp >37 5 C)	8	
Dyspnea	16	
Consciousness	3 5 16	unconscious somnolent awake but drowsy

Neutral Leo R I ® subcutaneously for at least 24 hours 3) Simultaneous infusion of 12 5 mEq potassium every hour for the first 4–6 hours then adapted to the plasma potassium 4) Bicarbonate supply 90–180 mmol as *sodium bicarbonate infusion was given in the event of* persistent metabolic acidosis after 2–8 hours of insulin treatment

RESULTS

Hyperglycemia

The blood glucose on admission (Table II) varied from 14 to 95 mmol/l (mean 33 6±18 4) being above 50 mmol/l in 4 cases A level of about 14 mmol/l was reached after 2–12 hours treatment (Fig 1) in most of the cases after 6–8 hours After the treatment had been changed to insulin subcutaneously the blood glucose was about 8 3 mmol/l, and only in a few cases as low as 3 3 mmol/l

An almost linear fall in blood glucose during low dose insulin treatment was observed especially when the change in blood glucose was expressed in per cent of the value at the start of insulin treatment The *hourly fall in blood* glucose (calculated as the slope of the regression lines) varied from 6 to 20% (mean 10 8%) of the initial value every hour

The dose of insulin used to obtain a blood glucose below 14 mmol/l averaged 50 IU (±20) and the total amount of insulin given during the first 24 hours 103 IU (mean±27) (Table III)

Table II *Laboratory findings on admission*

	n	Range	Mean	S D
Blood glucose (mmol/l)	4 11 9	50–95 25–49 14–24	33 6	18 4
Plasma total CO_2 (mmol/l)	6 15 3	≤10 10–20 >20	14	6
Plasma potassium (mEq/l)	9 8 7	≥5 0 3 6–4 9 <3 6	4 7	1 6
Plasma sodium (mEq/l)	24	110–154	133	8
Plasma creatinine (µmol/l)	24	115–548	274	106
Plasma osmolality (mosmol/l)	24	280–353	309	17
Plasma protein (g/l)	24	63–100	80	11
Hb (mmol/l)	24	6 1–11 5	8 7	1 4

Acidosis

The degree of metabolic acidosis was determined by measuring the total carbon dioxide in plasma *On admission all but 3 patients were acidotic (mean* 14±6 mmol/l) (Table II) In 6 patients the plasma carbon dioxide was below 10 mmol/l (Fig 2) After 12 hours of treatment with insulin acidosis had

Table III *Survey of 24 hours accumulated dose of insulin potassium, and fluid and the changes in blood glucose plasma potassium plasma creatinine and plasma osmolality (mean±S D)*

	Dose/ 24 h	Parameter 0 h	Parameter 24 h
Insulin (IU)	103±27		
Blood glucose (mmol/l)		33 7±18 4	9 6±3 7
Potassium (mEq)	151±70		
Plasma potassium (mEq/l)		4 7±1 6	3 8±0 7
Fluid (l)	8 9±2 6		
Plasma creatinine (µmol/l)		274±106	115±27
Plasma osmolality (mosmol/l)		309± 17	296± 9

Table IV *Change in plasma creatinine and plasma osmolality in 24 patients with diabetic coma*

	Hours after admission					
	0	4	8	12	16	24
Plasma creatinine (μmol/l)						
Range	115–548	80–522	71–318	80–212	71–168	70–177
Mean	274	230	168	141	115	106
S D	106	97	62	35	27	35
Plasma osmolality (mosmol/l)						
Range	280–353	281–345	273–329	279–322	278–324	283–312
Mean	309	305	298	296	296	296
S D	17	14	14	11	12	9

	Hours after admission				
	0–4	4–8	8–12	12–16	16–24
Fluid infusion rate (ml/h)					
Range	188–938	113–813	150–554	0–675	0–458
Mean	621	498	368	314	257
S D	173	200	140	148	202

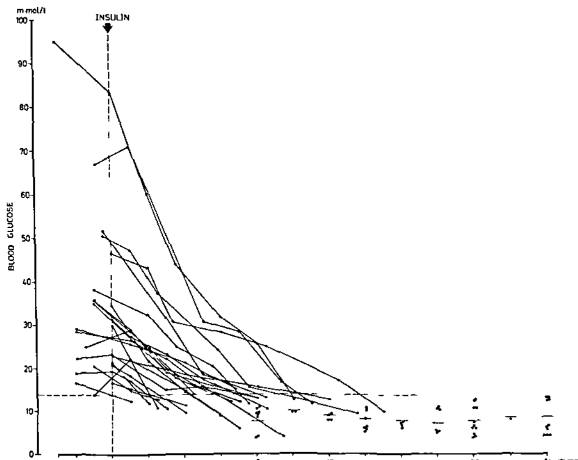


Fig 1 Change in blood glucose in 24 cases of hyperglycemia with and without ketoacidosis before and during 24 hours treatment with soluble insulin The individual values are connected until the blood glucose has declined below 14 mmol/l (horizontal dashed line) and are then marked every 4th hour until the final measured value after 20–24 hours

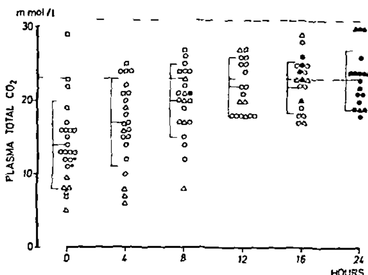


Fig 2 Individual values and mean+S D for plasma total CO_2 in all 24 cases at intervals of 4–8 hours A distinction is mad between values which at 0 hour were above or equal to 20 mmol/l (□) 10–20 mmol/l (○) or below 10 mmol/l (△) Every patient is then represented by this initial symbol until the last control in the 24-hour period (■ ● ▲) Six patients received a supply of bicarbonate (∗) — — =Normal limits of plasma total CO_2 (23–31 mmol/l)

been corrected (values ranging from 18 to 27 mmol/l) Six patients who showed persistent low plasma carbon dioxide concentrations of 5–14 mmol/l received after 2–8 hours treatment a small supply of bicarbonate 40–180 mmol resulting in rapid correction of the acidosis

Although acidosis was by and large corrected in all cases 10 patients still had plasma carbon dioxide below the lower normal limit (23 mmol/l) quite independently of the admission values (Fig 2)

Potassium

The initial plasma potassium values ranged from 2 3 to 8 7 mEq/l (mean 4 7±1 6) (Table II) Nine patients were hyperkalemic (>4 9 mEq/l) and 7 hypokalemic (<3 6 mEq/l) Despite infusion of 8–14 mEq potassium per hour the mean plasma potassium value decreased (Fig 3) although none of the patients presented with ECG changes characteristic of hypokalemia After 24 hours 15 of the patients had normal plasma potassium while 9 were hypokalemic Of these 5 had been hypokalemic 2 normokalemic and 2 hyperkalemic initially

A total of 75–280 mEq potassium (mean 151±70) (Table III) were infused during the first 24 hours An oral supply of potassium was given for the next week

Fluid balance

On admission the patients were characterized by marked dehydration elevated plasma creatinine plasma protein and Hb (Table II) The calculated plasma osmolality was above normal in 8 patients (Table IV) but only one had regular non ketotic hyperosmolar coma (353 mosmol/l) Plasma creatinine and plasma osmolality normalized gradually during treatment with insulin and rehydration with saline (Table IV)

With an hourly infusion (Table IV) of about 600 ml in the first 8 hours and 300 ml in the next 16 hours the total amount of fluids infused was 5–13.5 l (mean 8 9±2 6) (Table III) during 24 hours The 24 hour diuresis averaged only 3.3 l (±2 0) but none of the patients became cardially incompensated

Mortality

One patient died 8 hours after admission At this point he was neither hyperglycemic nor ketoacidotic (blood glucose 13 mmol/l total CO_2 21 mmol/l plasma potassium 5 1 mEq/l) He had been a diabetic for 20 years and had several late diabetic complications (cerebral and cardial atherosclerosis nephropathy proliferative retinopathy neuropathy and amputated right leg) The autopsy showed large posterior myocardial infarction The other patients reached their habitual condition after 1–4 days

DISCUSSION

In the treatment of acute diabetic dysregulation the low-dose insulin regimen either as continuous infusion or as hourly i m injections seems to predominate not only in adult diabetics (7 5 8 10 12 17 19 22) but also in children (3 4 16) Comparisons between the high and low-dose principles have been made in prospective studies (11 13 18) which consistently show that the risk of transient hy

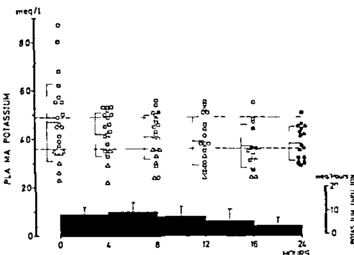


Fig 3 Individual values and mean±S D for plasma potassium at intervals of 4–8 hours in all 24 cases during 24 hours treatment □=Patients with initial plasma potassium above the normal limits (3 6–4 9 mEq/l marked by dashed lines) ○=patients with values within the normal limits Δ=patients with values below the normal limits The initial symbol follows the patient until the final control in the 24-hour period (■ ● ▲) The average rate of potassium infusion (mEq/h±S D) in 4–8-hour periods is shown at the bottom

poglycemia and hypokalemia is significantly reduced by giving insulin in small doses In a similar study however Soler et al (21) found a delayed correction of acidosis hyperglycemia and hypokalemia in their low-dose group This difference may easily be explained by the fact that the hyperglycemia and electrolyte disturbances were more pronounced in this group (1)

We chose the low-dose insulin regimen for the following reasons It satisfies the need for insulin as demonstrated by Sönksen et al (23) Large fluctuations in serum insulin and blood glucose are avoided together with the risk of late hypoglycemia The great deficiency of potassium is treated from the beginning It permits the elaboration of a simple protocol for a standardized treatment during the first 24 hours

Many authors prefer the continuous infusion of small doses (7 8 12 17 19) Compared with the i m administration the infusion of insulin results in a more constant insulin concentration in blood and in a more linear decline in blood glucose However as documented in this presentation the i m regimen is simple safe and efficient very easy to perform for unexperienced doctors in the emergency ward and the present results do not support doubts about the effectiveness of the low dose regimen in severely hyperglycemic and acidotic cases (9 14 15 20)

It should be pointed out that the potassium supply suggested by Alberti et al (2) may not be sufficient to normalize plasma potassium in all cases Therefore we have changed our regimen and increased the potassium infusion to 20 mEq every hour in patients with initial plasma potassium levels below 5 0 mEq/l Infusion of 12–13 mEq potassium per hour is still used when the plasma potassium is above this limit This change has also been made by Alberti (personal communication)

Table V *Changes in plasma potassium in a 42 year-old male diabetic during potassium infusion in the first 24 hours*

	Hours					
	0	2	6	9	16	22
Plasma potassium (mEq/l)	3 5	2 9	3 6	3 7	3 1	3 6
Infused potassium mEq/h		20	25	13	13	8
mEq/24 h			372			

ADDENDUM

Recently we had the opportunity to treat a 42 year-old man with previously undiagnosed diabetes with 20 mEq potassium every hour Despite only a moderate ketoacidosis he had a large potassium deficiency Initial values Blood sugar 17 1 mmol/l plasma total carbon dioxide 15 mmol/l plasma potassium 3.5 mEq/l plasma sodium 139 mEq/l plasma creatinine 190 μmol/l The changes in plasma potassium during the potassium infusion in the first 24 hours are shown in Table V In spite of the heavy doses the plasma potassium was kept only just within the normal range

REFERENCES

1 Alberti K G M M Comparison of different insulin regimens in diabetic ketoacidosis Lancet 1 83 1976
2 Alberti K G M M Hockaday T D R & Turner R C Small doses of intramuscular insulin in the treatment of diabetic coma Lancet 2 515 1973
3 Amato R S S Management of ketoacidosis in children N Engl J Med 294 1405 1976
4 Baum J D Jenkins P & Aynsley-Green A Immediate metabolic response to a low dose of insulin in children presenting with diabetes Arch Dis Child 80 373 1975
5 Efendić S & Luft R Behandling av svår diabetisk ketoacidos med eller utan koma Lakartidningen 72 5139 1975
6 Eskildsen P C & Nerup J Lav-dosis insulinbehandling af diabetisk ketoacidose Ugeskr Laeger 138/25 1499 1976
7 Ghirlanda G Rebuzzi A G Altomonte L Manna R Di Mauro P & Greco A V Insulin in the management of diabetic ketoacidosis Lancet 1 419 1976
8 Hannan T J & Stathers G M Constant low dose insulin infusion in severe diabetes mellitus Med J Aust 1 11 1976
9 Holt S & Cudworth A G Insulin resistance during continuous insulin infusion Lancet 1 44 1976
10 Jackson W P V & Vinik A I Modern methods of management of diabetic coma and precoma S Afr Med J 49 1869 1975
11 Kanter Y & Bessman A N Small versus large doses of insulin in diabetic ketoacidosis West J Med 122 509 1975
12 Kidson W Casey J Kraegen E & Lazarus L Treatment of severe diabetes mellitus by insulin infusion Br Med J 2 691 1974
13 Kitabchi, A E Treatment of diabetic ketoacidosis N Engl J Med 295 113 1976
14 Madison L L Low dose insulin A plea for caution N Engl J Med 294 393 1976
15 Menz W F Insulin infusion in diabetic coma Lancet 1 1182 1973
16 Moseley J Diabetic crises in children treated with small doses of intramuscular insulin Br Med J 1 59 1975
17 Page M McB Alberti K G M M Greenwood R Gumaa K A Hockaday T D R Lowy C Nabarro J D N Pyke D A Sonksen P H Watkins P J & West T E T Treatment of diabetic coma with continuous low-dose infusion of insulin Br Med J 2 687 1974
18 Quibrera R Nava M De Leon E D & Vidales M Treatment of diabetes ketoacidosis hyperosmolar coma and severe diabetes with low i v intermittent doses of insulin Rev Invest Clin 28 1 1976
19 Semple P F White C & Manderson W G Continuous intravenous infusion of small doses of insulin in treatment of diabetic ketoacidosis Br Med J 2 694 1974
20 Shaw K M Tillyer C R Morgan P G M & Bloom A Small doses of intramuscular insulin in the treatment of diabetic coma Lancet 1 1115 1974
21 Soler N G Fitzgerald M G Wright A D & Malins J M Comparative study of different insulin regimens in management of diabetic ketoacidosis Lancet 2 1221 1975
22 Sonksen P H Diabetic ketoacidosis N Engl J Med 291 1034 1974
23 Sonksen P H Tompkins C V Srivastava M C & Nabarro J D N Growth hormone and cortisol responses to insulin infusion in patients with diabetes mellitus Lancet 2 155 1972

Acta Med Scand 202 301–304 1977

Cerebrospinal Fluid Sorbitol and Myoinositol in Diabetic Polyneuropathy

Carita Servo Lea Bergstrom and Rainer Fogelholm

From the Fourth Department of Medicine and the Department of Neurology University of Helsinki Helsinki Finland

ABSTRACT Changes in cerebrospinal fluid (CSF) concentrations of sorbitol and myoinositol in 21 patients with diabetic polyneuropathy were studied with gas–liquid chromatography The sorbitol concentration was significantly increased in diabetic patients with elevated plasma glucose Myoinositol concentration was significantly decreased in patients with polyneuropathy compared with the controls Both alterations in polyol concentrations of the CSF were present already two months from onset of symptoms of diabetes Patients with peripheral polyneuropathy receiving oral hypoglycemic drugs did not have elevated plasma glucose and CSF sorbitol levels, but showed significantly decreased CSF myoinositol concentrations compared with the controls These observations suggest that myoinositol concentration may be decreased in the central nervous system in adult onset mild diabetes with normal plasma glucose and that the decrease in the myoinositol in CSF possibly is connected with the development of neuropathy

Alterations in the sorbitol pathway (5) and in myoinositol metabolism have both been associated with complications of diabetes (8)

The increase in sorbitol concentration in the lens has been associated with the development of diabetic cataract (6) and the increase in sorbitol concentration in nerve cord with the development of neuropathy in diabetic rats (4 5 7) In human diabetics the sorbitol concentration is increased in cerebrospinal fluid (CSF) compared with healthy controls (1 12 13) The development of impaired motor nerve conduction velocity (MNCV) in sciatic nerve of streptozoin diabetic rats is associated with a decrease in nerve free myoinositol concentrations compared with nerves from normal rats (8) We have noted increased sorbitol concentrations in the CSF of human diabetics compared with non diabetic controls (12 13)

In the present study we correlate signs of diabetic polyneuropathy to the concentrations of sorbitol and myoinositol in CSF and to the plasma glucose levels in diabetic patients

STUDY POPULATION AND METHODS

Diabetic patients 21 with polyneuropathy and five with normal nerve status were studied All five patients with normal nerve status were receiving oral hypoglycemic drugs Of the patients with polyneuropathy five were on insulin eleven on oral hypoglycemic drugs and one had carbohydrate restriction Four patients with neuropathy were studied at the time of hospitalization when the diagnosis of diabetes was established and before initiation of adequate treatment These four patients had carbohydrate restriction but no other treatment at the time of the study

None of the patients studied were ketotic Plasma glucose levels varied from 3 5 to 20 0 mmol/l and daily glucose excretion from zero to 660 mmol/24 hours The hyperglycemia was regarded as uncontrolled if fasting plasma glucose level was above 15 mmol/l and the urinary excretion more than 110 mmol/24 hours

The results were compared with those of 27 controls Careful clinical examination excluded metabolic cerebrovascular and neurological diseases in all controls None of them was on any kind of drugs

All diabetic patients were examined for signs of sensory and/or motor peripheral neuropathy The history most often included tingling and paresthesia of the feet and sometimes the patients exhibited foot drop Positive signs of neuropathy were loss of deep reflexes in the lower extremities absence of vibratory sensibility in the lower extremities and diminished cutaneous sensibility A neurophysiological examination including electromyography and conduction velocity measure ...er formed in 23 of the diabetic patients

Table I *Concentrations of sorbitol and myoinositol in cerebrospinal fluid of diabetic patients and healthy controls (median and range)*

Patients	Duration of diabetes	Plasma glucose (mmol/l)	CSF sorbitol (μM/l)	CSF myoinositol (μM/l)
Controls (n=27)		4 0 (3 2–5 0)	24 (14–38)	184 (126–236)
Diabetics without *neuropathy* (n=5)	2 (2–3) y	4 1 (3 5–7 0) n s	32 (15–40) n s	144 (100–180) n s
Diabetics with neuropathy (n=21)	2 (2–22) y	7 0 (3 5–20 0)**	45 (15–144)**	120 (45–150)**
Patients with neuropathy grouped according to treatment of diabetes				
On insulin				
Age 50	22 y	5 3	15	150
64	20 y	7 1	45	150
68	6 y	16 0	68	140
27	7 y	17 0	70	120
50	14 mo	20 0	144	120
Receiving oral hypoglycemic drugs				
Age 70	2 y	3 5	45	45
70	4 y	3 8	20	125
45	2 y	4 3	20	125
68	2 y	4 8	20	70
46	2 y	5 3	20	140
53	4 y	5 5	20	95
55	7 y	5 5	35	130
53	5 y	9 9	40	89
63	3 y	12 0	20	120
47	12 y	13 4	64	120
43	10 y	14 0	70	114
With carbohydrate restriction at the time of hospitalization				
Age 55	22 mo [a]	5 7	55	77
30	2 mo [a]	13 0	65	70
30	3 mo [a]	15 0	55	60
30	4 mo [a]	16 0	80	90
54	2 y	3 6	40	77

[a] Estimated duration of symptoms
Significant difference between test group and controls **p<0 01 n s =not significant

The muscle action potentials were recorded by concentric needle electrodes The spontaneous activity and pattern of full effort were recorded with a storage oscilloscope The motor conduction was measured in n peroneus profundus between capitulum fibulae and the ankle and in n medianus and/or n ulnaris between cubitale and the wrist or sulcus ulnaris and the wrist The sensory nerve conduction velocity (SNCV) was measured in the sural and radial nerves

The normal lower limits for SNCV and MNCV are sensory nerves n radialis 44 m/s n suralis 40 m/s motor nerves *n ulnaris 47 m/s n peroneus profundus 40 m/s*

Laboratory methods

Heparinized venous blood and 2 ml of CSF obtained by lumbar puncture were taken after overnight fasting The samples were immediately centrifuged Plasma glucose level was determined with a Beckman glucose analyzer

Samples of 100 μl CSF were prepared for gas-liquid chromatography The samples were diluted in 400 μl distilled water Glucose and fructose were removed by adding 4 5 μg hexokinase (Boehringer 15431) to each sample The hexose free samples were deproteinized with perchloric acid and neutralized with ion resin mixed bed (Amberlite resin IR 120 CH® and FF–IP SRA Permutit®) The neutral suspensions were dried in vacuum and acetylated Details of the method have been described elsewhere (12 13)

Statistics

The significance of difference between two groups was calculated with the Mann Whitney U test for non parametric statistics (1 4) Correlations were calculated as *linear regression coefficient* r

RESULTS

The concentrations of CSF sorbitol and myoinositol and the plasma glucose levels were altered in patients with diabetic polyneuropathy compared with the levels of controls and diabetic patients with normal nerve status (Table I)

Table II *Sensory (SNCV) and motor nerve (MNCV) conduction velocities and action potentials (EMG recordings) and the concentrations of sorbitol and myoinositol in cerebrospinal fluid (CSF) of diabetic patients with peripheral polyneuropathy*

p=pathologic (decreased conduction velocity fibrillation potentials) n=normal

Pat no	Treatment	Duration of diabetes	SNCV	MNCV	EMG	CSF sorbitol (μmol/l)	CSF myoinositol (μmol/l)
Patients studied before treatment of diabetes							
1	–	11 mo	p	n	n	55	77
2	–	4 mo	p	n	n	80	80
3	–	3 mo	p	n	n	55	60
4	–	2 mo	p	n	n	65	70
Range						(55–80)**	(60–80)
Patients with uncontrolled hyperglycemia and severe neuropathy							
1	Oral	10 y	p	p	p	70	114
2	Insulin	7 y	p	p	p	70	120
3	Insulin	6 y	p	p	n	68	140
4	Insulin	1 y	p	p	p	114	120
Range						(68–114) *	(114–140)
Patients with well controlled stable diabetes							
1	Insulin	22 y	p	n	n	15	150
2	Insulin	20 y	p	n	n	45	150
3	Oral	2 y	n	n	n	32	180
Range						(15–45) *n s*	(150–180) *n s*
Normal range						10–38	126–236

$^*p<0.05$ $^{**}p<0.01$ n s =not significant

As all patients with normal nerve status received oral hypoglycemic drugs the patients with polyneuropathy were further divided into four subgroups according to treatment (Table I) Plasma glucose levels and the CSF sorbitol concentrations were increased in patients on insulin and in patients studied before adequate treatment of diabetes but the patients receiving oral hypoglycemic drugs had normal plasma glucose levels and the CSF sorbitol concentrations did not differ significantly from the controls The CSF myoinositol concentration on the other hand was within the limits of the controls in patients without polyneuropathy but significantly decreased in all groups of patients with polyneuropathy (Table I) Patients with neuropathy on oral treatment had a lower median value of myoinositol than those on insulin (Table I) The alterations in the sorbitol and myoinositol concentrations were present at the time of hospitalization

Neurophysiological findings and alterations in polyol concentrations

Six of 21 patients had only impaired SNCV in 12 both SNCV and MNCV were impaired Three patients had impaired nerve conduction velocities and fibrillation potentials

All four patients studied at the time of hospitalization had decreased SNCV (Table II) MNCV and action potentials were normal The patients also had elevated plasma glucose levels significantly increased CSF sorbitol and significantly decreased CSF myoinositol concentrations

The four patients with the most severe electromyographical signs of polyneuropathy all had uncontrolled hyperglycemia and significantly altered polyol concentrations in the CSF

Only sensory peripheral polyneuropathy was found on the other hand in three patients with normal plasma glucose levels no glucosuria and a stable type of diabetes (in spite of a duration of 2–22 years) (Table II) These patients also had CSF myoinositol concentrations within the limits of the controls

DISCUSSION

The present findings that the sorbitol concentration in CSF of diabetic patients was significantly increased and that this increase was associated

the increase in plasma glucose levels are in agreement with our previous results (12 13) and also with the theory that sorbitol is synthetized from glucose in nervous tissue and that the synthesis is increased as the result of hyperglycemia (4 5)

In the present study the concentration of myoinositol in CSF was significantly decreased in diabetic polyneuropathy compared with controls Patients with neuropathy receiving oral hypoglycemic drugs had lower myoinositol concentrations in CSF than patients on insulin (Table I)

The concentration of intracellular free myoinositol decreases in the beginning of streptozoin diabetes in rats (8) simultaneously with the impairment of MNCV Adequate insulin therapy of diabetes or oral administration of myoinositol to the rats both seem to postpone or prevent the decrease in free myoinositol of nerve cells parallel to the impairment of MNCV

The concentrations of myoinositol are low in plasma compared with those in CSF (13) The intracellular concentration of myoinositol is high in glia cells (3, 10) CSF equilibrates more readily with brain tissue than with plasma Lipid soluble substances including alcohols easily penetrate the brain–CSF barrier (2) Therefore it seems likely that the myoinositol in CSF originates from intracellular free myoinositol in the central nervous system (CNS) The results suggest that diabetic polyneuropathy may be associated with low concentrations of myoinositol in the CNS Patients receiving oral hypoglycemic drugs may have altered myoinositol concentrations in CNS in spite of normal plasma glucose levels

Impaired SNCV appears early in diabetes (11) We observed decreased SNCV within two months from onset of symptoms indicating a disturbed glucose metabolism The sorbitol and myoinositol concentrations were significantly altered in CSF of four patients at the time of hospitalization when the diagnosis of diabetes was verified The results show that alterations in the polyol concentrations of CSF and impaired SNCV appear simultaneously within a few months from onset of disturbed glucose metabolism

The diabetic patients with normal myoinositol concentrations had less severe neuropathy than patients with significantly decreased myoinositol level in the CSF Neither alterations in polyol concentrations nor the findings of neuropathy correlated to the duration of the disease

The present study suggests that the metabolic disturbance in diabetes causes alterations in the sorbitol and myoinositol concentrations of CNS which also alter polyol concentrations in the CSF The alterations can probably be avoided with adequate control of diabetes

REFERENCES

1 Aloia J F & Nilakantan S The cerebrospinal fluid in uncontrolled diabetes mellitus Am J Med Sci 266 3 203 1973
2 Dawson H Physiology of the cerebrospinal fluid pp 86–87 Churchill London 1967
3 Dawson R M C & Freinkel N The distribution of free mesoinositol in mammalian tissues including some observations on the lactating rat Biochemistry 78 606 1961
4 Gabbay K H Factors affecting the sorbitol pathway in diabetic nerve Diabetes (Suppl) 1 336 1969
5 — The sorbitol pathway and complications of diabetes N Engl J Med 288 16 831 1973
6 Gabbay K H & Kinoshita J H Mechanism of development and possible prevention of sugar cataracts Isr J Med Sci 8 1557 1972
7 Gabbay K H Merola L O & Field R A The sorbitol pathway presence in nerve cord with substrate accumulation in diabetes Science 151 209 1966
8 Greene D A Pacifico V De Jesus P V Jr & Winegrad A I Effects of insulin and dietary myoinositol on impaired peripheral motor nerve conduction velocity in acute streptozoin diabetes J Clin Invest 55 1326 1975
9 Gregersen G Diabetic neuropathy Influence of age sex metabolic control and duration of diabetes on motor conduction velocity Neurology 17 972 1967
10 Kusama H M & Stewart M A Levels of myoinositol in normal and degenerating peripheral nerve J Neurochem 17 317 1970
11 Lamontagne A & Buchtal F Electrophysiological studies in diabetic neuropathy J Neurol Neurosurg Psychiatry 33 442 1970
12 Pitkänen E & Servo C Cerebrospinal fluid polyols in patients with diabetes mellitus Clin Chim Acta 44 437 1972
13 Servo C & Pitkanen E Variations in polyol levels in cerebrospinal fluid and serum in diabetic patients Diabetologia 11 575 1975

Acta Med Scand 202 305–306 1977

Carnitine Concentration in Skeletal Muscle Tissue from Patients with Diabetes Mellitus

G Cederblad K Lundholm and T Schersten

From the Department of Clinical Chemistry and the Surgical Metabolic Research Laboratory Sahlgren s Hospital University of Goteborg Göteborg Sweden

ABSTRACT L Carnitine concentration was determined in vastus lateralis and abdominal rectus muscle tissue from 15 patients with diabetes mellitus and 66 controls Nine of the diabetics were treated with diet and hypoglycemic drugs only and six with insulin The carnitine concentration was determined enzymatically with labeled [1 ^{14}C] acetyl-coenzyme A as a substrate and given per weight of non-collagen protein The concentration in muscle tissue did not differ significantly between patients and controls Patients with insulin treated diabetes had the same concentration of carnitine in muscle tissue as those treated with hypoglycemic drugs The drastic decreases in carnitine muscle concentration and in carnitine body pool seen in alloxan-diabetic rats are not observed in skeletal muscle of diabetic humans

The carnitine (L 3 hydroxy-4 N trimethylaminobutyrate) concentration is high in tissues with high fatty acid oxidation i e in heart and skeletal muscle tissue According to Mehlman et al (4 5) the body pool of carnitine in alloxan-diabetic rats (mg carnitine/100 g b wt) is decreased to less than one third and the turnover time to one fourth of that of controls These authors also reported that insulin increased the body pool of carnitine but not to the level of controls These results suggest that insulin may play a role in the regulation of the body pool of carnitine The carnitine concentration in serum is normal in human diabetes (1) However this cannot be taken as an indication that the body pool of carnitine is normal

The serum carnitine in man has been estimated to be only 1/1 000 of the skeletal muscle pool and no correlation has been found between carnitine concentration in plasma and skeletal muscle tissue in normal subjects (3) In the present study the carnitine concentration was determined in skeletal muscle tissue from 15 diabetic patients one of whom was in a poorly controlled state

STUDY POPULATION AND METHODS

The patient group comprised eight men and seven women 71±9 (mean±S D) years old with diabetes of the maturity onset type Nine patients were treated with diet and hypoglycemic drugs and six with insulin Seven patients had severe complications such as gangrene in the lower extremities four had intermittent claudication and four had no sign of severe diabetic complication The control group consisted of 66 students and patients operated on for varicose vein disease or uncomplicated gallstone disease 43±18 (mean±S D) years old (3)

The muscle biopsy specimens were taken from the lateral vastus muscle of the leg and always at a distance from the region with impaired blood flow in some patients from the abdominal rectus muscle Surgical procedures and biopsy handling were performed as described previously (3) Informed consent was obtained from all patients Perchloric soluble carnitine was determined enzymatically using labeled [1 ^{14}C] acetyl-CoA as a substrate (2)

Wilcoxon s two-sample test was used to test the statistical significance

RESULTS AND DISCUSSION

The carnitine concentration in leg muscle tissue or abdominal muscle tissue from patients with diabetes was not significantly different from that of appropriate controls (Fig 1) The median value in abdominal and in leg muscle tissue from the diabetics was 20 2 and 27 5 μmoles/g non-collagen protein respectively The corresponding values for the controls were 20 7 and 25 0 μmoles/g non-collagen protein The carnitine concentration in muscle tis

'0–77'984

the increase in plasma glucose levels are in agreement with our previous results (12 13) and also with the theory that sorbitol is synthetized from glucose in nervous tissue and that the synthesis is increased as the result of hyperglycemia (4 5)

In the present study the concentration of myoinositol in CSF was significantly decreased in diabetic polyneuropathy compared with controls Patients with neuropathy receiving oral hypoglycemic drugs had lower myoinositol concentrations in CSF than patients on insulin (Table I)

The concentration of intracellular free myoinositol decreases in the beginning of streptozoin diabetes in rats (8) simultaneously with the impairment of MNCV Adequate insulin therapy of diabetes or oral administration of myoinositol to the rats both seem to postpone or prevent the decrease in free myoinositol of nerve cells parallel to the impairment of MNCV

The concentrations of myoinositol are low in plasma compared with those in CSF (13) The intracellular concentration of myoinositol is high in glia cells (3 10) CSF equilibrates more readily with brain tissue than with plasma Lipid soluble substances including alcohols easily penetrate the brain–CSF barrier (2) Therefore it seems likely that the myoinositol in CSF originates from intracellular free myoinositol in the central nervous system (CNS) The results suggest that diabetic polyneuropathy may be associated with low concentrations of myoinositol in the CNS Patients receiving oral hypoglycemic drugs may have altered myoinositol concentrations in CNS in spite of normal plasma glucose levels

Impaired SNCV appears early in diabetes (11) We observed decreased SNCV within two months from onset of symptoms indicating a disturbed glucose metabolism The sorbitol and myoinositol concentrations were significantly altered in CSF of four patients at the time of hospitalization when the diagnosis of diabetes was verified The results show that alterations in the polyol concentrations of CSF and impaired SNCV appear simultaneously within a few months from onset of disturbed glucose metabolism

The diabetic patients with normal myoinositol concentrations had less severe neuropathy than patients with significantly decreased myoinositol level in the CSF Neither alterations in polyol concentrations nor the findings of neuropathy correlated to the duration of the disease

The present study suggests that the metabolic disturbance in diabetes causes alterations in the sorbitol and myoinositol concentrations of CNS which also alter polyol concentrations in the CSF The alterations can probably be avoided with adequate control of diabetes

REFERENCES

1 Aloia J F & Nilakantan S The cerebrospinal fluid in uncontrolled diabetes mellitus Am J Med Sci 266 3 203 1973
2 Dawson H Physiology of the cerebrospinal fluid pp 86–87 Churchill London 1967
3 Dawson R M C & Freinkel N The distribution of free mesoinositol in mammalian tissues including some observations on the lactating rat Biochemistry 78 606 1961
4 Gabbay K H Factors affecting the sorbitol pathway in diabetic nerve Diabetes (Suppl) 1 336 1969
5 — The sorbitol pathway and complications of diabetes N Engl J Med 288 16 831 1973
6 Gabbay K H & Kinoshita J H Mechanism of development and possible prevention of sugar cataracts Isr J Med Sci 8 1557 1972
7 Gabbay K H Merola L O & Field R A The sorbitol pathway presence in nerve cord with substrate accumulation in diabetes Science 151 209 1966
8 Greene D A Pacifico V De Jesus P V Jr & Winegrad A I Effects of insulin and dietary myoinositol on impaired peripheral motor nerve conduction velocity in acute streptozoin diabetes J Clin Invest 55 1326 1975
9 Gregersen G Diabetic neuropathy Influence of age sex metabolic control and duration of diabetes on motor conduction velocity Neurology 17 972 1967
10 Kusama H M & Stewart M A Levels of myoinositol in normal and degenerating peripheral nerve J Neurochem 17 317 1970
11 Lamontagne A & Buchtal F *Electrophysiological* studies in diabetic neuropathy J Neurol Neurosurg Psychiatry 33 442 1970
12 Pitkanen E & Servo C Cerebrospinal fluid polyols in patients with diabetes mellitus Clin Chim Acta 44 437 1972
13 Servo C & Pitkanen E Variations in polyol levels in cerebrospinal fluid and serum in diabetic patients Diabetologia 11 575 1975

Acta Med Scand 202 307–311 1977

Serum Inorganic Phosphate in Middle-Aged Men

I Inverse Relation to Body Weight

Folke Lindgarde and Erik Trell

From the Department of Medicine and the Section of Preventive Medicine University of Lund Malmo General Hospital Malmo Sweden

ABSTRACT Serum inorganic phosphate was determined in 752 men born in 1926 An inverse correlation was found between serum inorganic phosphate levels and body weight Other parameters of possible relevance to this finding, such as serum calcium serum albumin, serum γ glutamyltransferase and the occurrence of upper gastrointestinal disorders were analysed and no correlations to the phosphate level were detected

Serum calcium is considered to be one of the most narrowly regulated physiological constants in man (19) This constancy seems to be necessary for many biological processes such as neuronal excitability muscle contraction membrane permeability and hormone release In contrast to the stability of serum calcium which seems to be the result of the interplay between vitamin D parathyroid hormone and calcitonin (24) the wider normal range of serum inorganic phosphate (11 12) is noteworthy considering that these hormones are also involved in the phosphorus balance (5 6 22) Various other hormones such as insulin (4) and growth hormone (3) may influence the level of serum inorganic phosphate An increase in body fat is accompanied by several hormonal changes Insulin concentrations seem to be positively correlated to the relative body weight (21) The level of growth hormone is decreased and its responsiveness to provocative stimulation reduced in obesity (7) However no studies have been made to determine whether the levels of serum calcium and serum inorganic phosphate are correlated with body weight

Phosphorus plays a vital role in the metabolism of all living cells Clinical evidence for a phosphorus depletion syndrome in man characterized by anorexia and weakness has been reported after prolonged antacid ingestion (17) and in chronic alcoholics (26) The purpose of the present study is to examine in an age uniform male population where possible age and sex influences are constant *a*) correlations between serum inorganic phosphate serum calcium and body weight parameters and *b*) factors prevalent in a middle aged male population such as alcoholic abuse and upper gastrointestinal disease possibly linked with antacid ingestion which may influence the serum inorganic phosphate levels

STUDY POPULATION

All men born in 1926 and living in Malmo in the autumn of 1974 (n=1 560) were identified from the population records and invited to a medical screening examination at the Section of Preventive Medicine Department of Internal Medicine Malmö General Hospital between Sept 1974 and Sept 1975 A total of 1 126 men (71 %) responded to the invitation and form the population basis of the present investigation A medical questionnaire weight and height measurements serum calcium (S Ca) serum albumin (S Alb) and serum γ glutamyltransferase (S GT) analyses and a glucose tolerance test were included in the screening examination performed in all individuals

Serum inorganic phosphate (S P) was obtained in a subsample of the 1 126 individuals which was collected at random each day and on which an extensive set of laboratory analyses was performed to serve as a continuous normal reference material for the Department of Clinical Chemistry Malmö General Hospital This subsample which at the conclusion of the year class investigation amounted to a total of 752 men born in 1926 constitutes our study population

METHODS

Analytical methods

All investigations were performed in the morning after an overnight fast All blood samples were obtained by veni-

Table I *Mean values and S D of weight A/I weight S Ca S P and S Alb in the whole study population (n=752)*

	Mean	S D
Weight (kg)	77 130	11 099
A/I weight	1 083	0 137
S Ca (mEq/ml)	4 785	0 175
S P (mg/100 ml)	2 752	0 464
S Alb (mg/100 ml)	4 676	0 274

puncture in the supine position after 10 min rest Weight and height were measured by routine techniques A relative weight index according to the formula actual (measured) weight/ideal weight for body height (A/I weight) (9) was calculated for each individual The tables of Lindeberg et al (14) were used as ideal weight reference

S P S Ca S Alb and S-GT were analysed by standard methods at the Department of Clinical Chemistry, Malmo General Hospital At the time of study the SI metric system was not yet being applied Therefore the values of S P are expressed in mg/100 ml S Ca in mEq/l S Alb in mg/100 ml and S-GT in U/l (upper normal limit 65 U/l)

Statistical methods

Calculations of mean values and S D were done according to routine computerized or semicomputerized statistical methods as were least square linear regression analyses and determinations of correlation coefficients Student s *t* test was used when calculating the significance levels of differences between groups

RESULTS

Table I shows mean values and S D of weight A/I weight S P S Ca and S Alb in the whole study population In terms of the A/I weight index there is on the whole a slight overweight tendency There was one S P value of 1 3 and one of 4 6 mg/100 ml otherwise the S P range in the material was 1 5–4 0 mg/100 ml No case of hyperparathyroidism or hypoparathyroidism was found

Table II gives paired correlation coefficients and significance levels of the correlations between weight and A/I weight weight and S Ca weight and S P A/I weight and S Ca A/I weight and S P and S Ca and S P Obviously there is a highly significant correlation between weight and A/I weight Neither of these is significantly correlated to S Ca However both weight ($r=-0\ 122$ $p<0\ 001$) and still more evident A/I weight ($r=-0\ 144$ $p<0\ 001$) exhibit a highly significant inverse correlation to the S P level while there was no significant association between S Ca and S P

In Table III all subjects in the study group with S P≤2 0 mg/100 ml are compared with all subjects with S P≥3 5 mg/100 ml This division representing polar phosphate low and phosphate high subgroups of the population is symmetric around the mean S P value in the whole study population but is somewhat extended over the fifth decentile on both sides of the distribution in order to obtain a sizeable number of cases Mean and S D of A/I weight and of S Ca are compared in these subgroups There is a significantly higher ($p<0\ 001$) A/I weight in the phosphate low subgroup (A/I weight 1 137) than in the phosphate high subgroup (A/I weight 1 008) while there is no significant difference between the S Ca values Table III also compares mean values and S D of S Alb and S GT in the two polar S P groups and no significant correlation is found We also checked the whole material for correlations in linear regression analysis between S Alb and S P and found no significant association ($n=752$ $r=-0\ 091$ N S), while there was a highly significant correlation between S Alb and S Ca ($n=752$ $r=0\ 356$)

The relation between S P and body weight in our study population is further illustrated in Fig 1 Here the material is divided into consecutive arbitrary S P subclasses of 1 5–1 9 2 0–2 4 2 5–2 9 3 0–3 4 and 3 5–3 9 mg/100 ml The mean value and S D of the actual weight and calculated ideal weight of the individuals in these subclasses are graphically represented There are no obvious differences in the calculated ideal weight (body height) in the different S P groups while the inverse relation between the S P level and body weight can be traced throughout the distribution

Finally we checked the medical questionnaires in

Table II *Correlation coefficients and p values of the paired relations in the total study population (n=752) between weight A/I weight S Ca and S P*

	Weight	A/I weight	S Ca
Weight	–		
A/I weight	$r=0\ 875$ $p<0\ 001$	–	
S Ca	$r=-0\ 008$ N S	$r=-0\ 003$ N S	–
S P	$r=-0\ 122$ $p<0\ 001$	$r=-0\ 144$ $p<0\ 001$	$r=-0\ 074$ N S

N S = not significant

Table III *Mean values S D and significance levels of the difference of A/I weight S Ca S Alb and S GT in serum phosphate low and serum phosphate high subgroups*

	S-P≤2 0 (n=48)		S P≥3 5 (n=43)		Significance of the difference
	Mean	S D	Mean	S D	
(S-P)	(1 848	0 162)	(3 691	0 223)	–
A/I weight	1 137	0 120	1 008	0 141	p<0 001
S-Ca (mEq/ml)	4 817	0 163	4 744	0 310	N S
S Alb (mg/100 ml)	4 704	0 281	4 626	0 292	N S
S-GT (U/l)	43 500	20 899	43 256	27 719	N S

the phosphate low and phosphate high subgroups in order to examine possible differences in the occurrence of previous or present gastrointestinal disorders We found no certain tendencies in this respect

DISCUSSION

Ionized calcium the physiologically active fraction of serum calcium amounts to about 46% of S Ca in normal subjects 40–60 years old (15) The S D of the ionized calcium in the above mentioned study was 0 20 mg/100 ml which amounts to about half the S D for S Ca in the present population The diffusible fraction i e the biologically most important component of S P is estimated to be 85% of the total S P level (16) The S D of S P in our

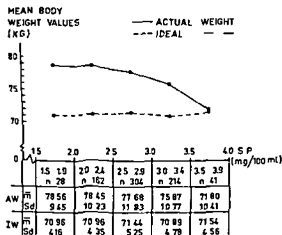


Fig 1 Actual and ideal weight in 28 cases with S P 1 5–1 9 mg/100 ml 162 with 2 0–2 4 304 with 2 5–2 9 214 with 3 0–3 4 and 41 cases with S P 3 5–3 9 mg/100 ml Mean values and S D of the actual weight (*AW*) and ideal weight (*IW*) in the consecutive S-P subgroups are also tabulated

material was 0 46 mg/100 ml which supports the view that the physiologically active calcium fraction is more narrowly regulated than the corresponding phosphate parameter

In adults there is an age dependency of the levels of serum calcium in both sexes (15) and of serum phosphate in males (11 12) In the present male population study where the age influence was kept constant there was no significant correlation between S P and S Ca It seems reasonable to assume that under normal metabolic conditions the hormonal regulation of S Ca does not influence the S P level to a noticeable degree Further evidence that the S P balance may be influenced by factors independent of the S Ca regulation might be supplied by the findings in our study that an increased body weight was associated with a tendency to lower S P levels but that no corresponding relation could be traced for S Ca

A large proportion of obese individuals both with normal and with mildly impaired carbohydrate intolerance show increased fasting insulin and glucose levels (9) During infusion of insulin there is a decrease in plasma phosphate but no trend to hypocalcemia (4) It is tempting to speculate that the higher insulin levels found in obese compared with non-obese individuals may influence the phosphate balance resulting in a tendency to hypophosphatemia However preliminary results from a study on the relation of basal insulin to serum phosphate levels do not support this theory (16)

In obesity an increase in the plasma volume has been suggested (2) and such a volume expansion might cause decreased S P levels If dilution is the explanation for the inverse relation between body weight and S P in our study population there would presumably be a similar effect upon S Alb levels and thus a relation between S Alb and S P How

ever neither in the study group as a whole, nor in the low and high S P subgroups (Table III), could an association between S Alb and S P be traced

According to Knochel et al (13) the majority of patients with severe alcoholism have only slightly depressed S P values but in the refeeding period during hospitalization the phosphate level falls sharply Rajan et al (23) have demonstrated that hepatocellular changes may occur during hypo phosphatemia According to the biochemical parameters used in the present study viz S GT and S Alb there were no indications of an over representation of liver disorders in the low com pared with the high S P group

Previous reports on hypophosphatemia caused by antacid ingestion have stressed the complaints of muscle weakness (17) We had no spontaneous re ports of such symptoms in the individuals belonging to our low S P group Neither did we find an over representation of upper gastrointestinal disorders possibly linked with a tendency to antacid ingestion in the low S P group compared with the high

The inverse relation between S P and body weight which our study indicates has to the best of our knowledge not been noted before The relation seems interesting and may warrant further investi gation From a hypothetical point of view at least three aspects may be considered

There are observations (10) supporting a lower total calory intake in moderate obesity than in indi viduals of normal body weight In middle aged American women there is a significant correlation between the overall composition of the diet and the retention of natrium calcium and phosphorus (20 25) It seems plausible therefore that one possible explanation of an inverse relation between body weight and S P might be a phosphorus deficient diet

In acromegaly high levels of S P are found When human growth hormone (HGH) is injected to homo the reabsorption of phosphate in the renal tubuli increases (3) In obese individuals low levels of HGH are found in the basal state as well as after stimulation for instance by physical exercise (8) The mechanism of such a suppression of HGH in obese individuals is unknown

During physical exercise S P levels significantly increase in spite of a concurrent marked elevation of the urinary phosphate excretion (1) Lean indi viduals are much more physically active than obese (18) It may be conjectured that higher and more frequent physical activity in lean individuals may result in an increased overall S P balance compared with obese individuals

In conclusion it is conceivable that a tendency to low S P in overweight men might reflect a metabolic adaption to low dietary phosphorus intake low physical activity or a hormonal imbalance

REFERENCES

1 Aurell M Carlsson M Grimby G & Hood B Plasma concentration and urinary excretion of certain electrolytes during supine work J Appl Physiol 22 633 1967
2 Chiang B N Perlmann L V & Epstein F H Overweight and hypertension A review Circulation 39 403 1969
3 Corvilain J & Abramov M Some effects of human growth hormone on renal hemodynamics and on tubular phosphate transport in man J Clin Invest 41 1230 1962
4 DeFronzo R A Cooke C R Andres R Faloona G R & Davis P J The effect of insulin on renal handling of sodium potassium calcium and phosphate in man J Clin Invest 55 845 1975
5 Deluca H F Recent advances in our understanding of the vitamin D endocrine system J Lab Clin Med 87 7 1976
6 Diaz Buxo J A & Knox F G Effects of para thyroid hormone on renal function Mayo Clin Proc 50 537 1975
7 El Khodary A Z Ball M F Stein B & Canary J J Effects of weight loss on the growth hormone response to arginine infusion in obesity J Clin En docrinol 32 42 1971
8 Hansen A P Serum growth hormone response to exercise in non obese and obese normal subjects Scand J Clin Lab Invest 31 175 1973
9 Hedstrand H Studies in preventive medicine with particular reference to detection and treatment of risk factors for cardiovascular disease Thesis Uppsala 1975
10 Johnson M L Burke B S & Mayer J Relative importance of inactivity and overeating in the energy balance of obese high school girls Am J Clin Nutr 4 37 1956
11 Keating F R Jones J D Elveback L R & Randall R V The relation of age and sex to distribu tion of values in healthy adults of serum calcium inorganic phosphorus magnesium alkaline phos phatase total proteins albumin and blood urea J Lab Clin Med 73 825 1969
12 Keating F R Jones J D & Elveback L R Dis tribution of serum calcium and phosphorus values in unselected ambulatory patients J Lab Clin Med 74 507 1969
13 Knochel J P Bilbrey G L Fuller T J & Carter N W The muscle cell in chronic alcoholism the possible role of phosphate depletion in alcoholic myopathy Ann NY Acad Sci 252 274 1975

14 Lindeberg W Natvig H Rygh A & Svendsen K Hoyde og vektsundersokelser hos voksne menn og kvinner Tidsskr Nor Laegeforen 76 361 1956
15 Lindgarde F Potentiometric determination of serum ionized calcium in a normal human population Clin Chim Acta 40 477 1972
16 Lindgarde F & Trell E Serum inorganic phosphate and serum calcium in middle aged men II Relation to glucose and insulin parameters in glucose tolerance tests Acta Med Scand To be published
17 Lotz M Zisman E & Bartter F C Evidence for a phosphorus-depletion syndrome in man N Engl J Med 278 409 1968
18 Mayer J Inactivity as a major factor in adolescent obesity Ann NY Acad Sci 131 502 1965
19 Mc Lean F C & Hastings A B The state of calcium in the fluids of the body J Biol Chem 108 285 1935
20 Ohlson M A Roberts P H Joseph S A & Nelson P M Dietary practices of 100 women from 40 to 75 years of age J Am Diet Assoc 24 286 1948
21 Olefsky J Reaven G M & Farquhar J W Effects of weight reduction on obesity J Clin Invest 53 64 1974
22 Queener S H & Bell N H Calcitonin A general survey Metabolism 24 555 1975
23 Rajan K S Levinson R & Leevy C M Hepatic hypoxia secondary to hypophosphatemia Clin Res 21 521 1973
24 Rasmussen H Bordier P Kurokawa K Nagata N & Ogata E Hormonal control of skeletal and mineral homeostasis Am J Med 56 751 1974
25 Roberts P H Kerr C H & Ohlson M A Nutritional status of older women Nitrogen calcium phosphorus retentions of nine women J Am Diet Assoc 24 292 1948
26 Territo M C & Tanaka K R Hypophosphatemia in chronic alcoholism Arch Intern Med 134 445 1974

Acta Med Scand 202 313–318 1977

Lactoferrin and Lysozyme in Arthritic Exudates

Jörgen Malmquist Jan I Thorell and Frank A Wollheim

From the Departments of Medicine and Nuclear Medicine and the Division of Rheumatology University of Lund Malmo General Hospital Malmo Sweden

ABSTRACT Lactoferrin (LF) has been assayed by radioimmunoassay in plasma and arthritic exudates and compared with lysozyme (LZ) levels and leukocyte counts The mean LF concentration in 38 rheumatoid arthritis (RA) exudates was 9 1 mg/l (range 0 02–39 2) In 30 non RA exudates LF was 3 3 mg/l (range 0 01–14 6) The corresponding LZ levels were 7 4 mg/l (range 2 5–18 5) in RA and 4 7 (range 1 0–12 5) in non RA fluids Exudate/plasma ratios were much higher for LF than for LZ and higher in RA than in non RA exudates whereas leukocyte counts did not differ The LF/leukocyte count ratio was significantly higher in RA than in the non RA group The data suggest a more prominent release of neutrophilic granulocyte components in RA than in non RA arthritis

Enzymes released from cells in the synovial lining or in the joint cavity are considered to have a major role in tissue injury in arthritis (12–15 18 22 23 31–33) Neutrophilic granulocytes present in inflammatory joint exudates are one possible source of such enzymes (26) Measurements of neutrophil components in exudates are used to demonstrate the occurrence of enzyme release and as indicators of disease activity

Lysozyme (LZ) concentrations in rheumatoid exudates are higher than in non inflammatory joint fluids and likewise higher than in plasma (21 24 25) However interpretation of exudate LZ levels is hampered by uncertainty about the origin of the enzyme In addition to neutrophilic granulocytes in the exudate various cell types in the synovial membrane (24) as well as articular cartilage (7 17) may release LZ

A substance of more specific neutrophil origin transcobalamine large (TCL) has been found in much higher concentration in joint fluid than in plasma indicating local release (8 9) Its determination however is too complicated for general use

Another relevant protein is lactoferrin (LF) present in the secondary (specific) granules of neutrophils (30) It is an iron binding protein which contributes to the redistribution of iron occurring in inflammatory conditions (29) LF has been demonstrated and measured in inflammatory synovial exudates (1 3 5)

In the present study a radioimmunoassay method has been employed for the measurement of joint fluid LF as a possible indicator of inflammatory activity The LF values have been evaluated together with LZ levels leukocyte counts and clinical data

STUDY POPULATION AND METHODS

The study population consisted of 52 unselected patients seen in the Arthritis Unit The diagnoses and number of specimens are shown in Table I The patients with rheumatoid arthritis (RA) were all seropositive and satisfied the criteria for classical" or definite" RA (27)

Blood and synovial fluid were collected in tubes containing EDTA Plasma was separated within an hour The synovial fluid was treated with hyaluronidase (from bovine testis Leo Pharmaceuticals Helsingborg Sweden) Two ml were incubated with 15 μl of a 1 mg/ml solution of the enzyme at 37°C for 30 min Subsequently an aliquot was taken for cell counting The remainder of the hyaluronidase treated synovial fluid was centrifuged at 12 000 *g* for 20 min The samples were stored at −20°C until assayed LZ was determined by the Laurell electroimmunoassay as described by Johansson & Malmquist (16) LF was measured with a radioimmunoassay as described in detail previously (11) In a ... dates with high LF concentrations the

Presented in part at a meeting of the Swedish Society for Rheumatology in Dec 1973

Table I *LF and LZ concentrations in exudates according to diagnosis*

Diagnosis	No of Pats	No of Exudates	Lactoferrin (mg/l) Mean	Lactoferrin (mg/l) Range	Lysozyme (mg/l) Mean	Lysozyme (mg/l) Range
RA	30	38	9 09	0 02–39 2	7 42	2 5–18 5
Non RA	22	30	3 30	0 01–14 6	4 70	1 0–12 5
Reiter s	5	9	2 51	0 02–9 3	4 17	1 5–8 5
Pyrophosphate synovitis	6	6	6 0	0 01–13 4	5 83	1 5–12 5
Chron oligoarthritis	3	4	7 18	0 8–14 6	6 63	3 5–9 5
Psoriatic arthropathy	2	3	1 39	0 41–2 75	3 84	3 0–5 0
Osteoarthrosis	2	4	0 75	0 14–1 8	2 38	1 0–3 0
Juvenile RA	1	1	0 52		7 5	
Dermatomyositis	1	1	0 25		3 5	
Stevens Johnson s	1	1	7 0		8 0	
Hyperlipidemia	1	1	0 20		4 0	

checked by radial immunodiffusion Satisfactory agreement between methods was found

Statistical methods

Relations between LF LZ and leukocyte counts in exudates were evaluated by linear regression analysis The Mann Whitney test was used to compare RA with non RA cases with regard to exudate/plasma ratios of LZ and LF exudate LF/leukocyte ratios and exudate LF/LZ ratios

RESULTS

Lactoferrin

Plasma concentrations of LF were 0 06–0 70 mg/l in RA and 0 07–0 67 mg/l in non RA patients (normal 0 13–0 42) (11)

In exudates LF levels were higher in the RA group (mean 9 0 mg/l range 0 02–39 2) than in the non RA group (mean 3 3 mg/l range 0 01–14 6) Concentrations above 10 mg/l occurred in 14/38 RA and 3/30 non RA samples (Fig 1) Leukocyte counts were similar in the two groups (mean 8 200 vs 8 400) A correlation between LF concentrations and leukocyte counts was found with $r=0$ 45 in the RA and 0 57 in the non RA group The exudate LF/cell count ratios were significantly higher in the RA than in non RA exudates mean $1\ 19\times10^{-3}$ vs $0\ 69\times10^{-3}$ $p<0$ 005

Lysozyme

Plasma LZ concentrations were 1 5–7 mg/l in RA and 1 5–5 5 mg/l in non RA patients i e only minor elevations above the normal range (1 0–4 5)

In the RA exudates the LZ concentrations ranged 2 5–18 5 mg/l (mean 7 2) and in non RA exudates 1 0–12 5 mg/l (mean 5 6) (Fig 2) Whereas a rather high correlation was found between LZ levels and leukocyte counts in the non RA group ($r=0$ 68) there was no significant correlation in the RA exudates ($r=0$ 18)

Relation between LF and LZ

In both patient groups there were significant correlations between exudate LF and LZ levels The highest LF/LZ ratios occurred in the RA group (Fig 2)

In order to obtain an improved indicator of intraarticular inflammatory activity the exudate/plasma ratios were calculated for LF and LZ In Fig 3 the ratios of individual exudate/plasma pairs have been joined by lines LF ratios exceeding 10 were observed in 32 of 38 RA specimens as compared to 11 of 29 non RA samples This difference is highly significant ($p<0$ 001) The LZ ratios also differed between the groups ($p<0$ 005)

Relation to clinical parameters

No relation was found between LF or LZ on the one hand and volume of effusion duration of synovitis or degree of joint destruction on the other among the RA patients In cases from which multiple exudates were examined these were similar in LF concentrations

Wide ranges of exudate LF concentrations were observed within different diagnostic groups (Table I)

DISCUSSION

In view of recent data on the importance of lysosomal enzyme release as a mediator in joint inflammation and possibly in joint destruction (15 18) markers of lysosomal function are attracting

Fig 1 Exudate leukocyte counts in relation to exudate lactoferrin concentrations (*ELF*) in RA and non RA exudates

increased interest In the present study the leukocyte granular component lactoferrin has been determined in exudates and plasma by a previously described radioimmunoassay (11) LF levels found in plasma are somewhat lower than those reported by Rumke et al (28) and by Bennett and Mohla (2) LF values in exudates have previously been given by Bennett and Skosey (3) Though their results are in general accordance with ours their exudate LF levels were considerably higher but no methodological details were included Some of these discrepancies may be due to differences between standards (e g degree of iron saturation) or to differences in antibody specificity of the various antisera

However one possible source of error is post sampling in vitro leakage from cells as shown by Rumke et al (28) In vitro release from intact leukocytes is calcium-dependent In the present study EDTA was used in all blood and exudate

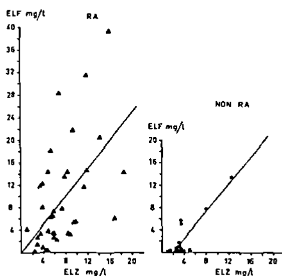


Fig 2 Exudate lactoferrin (*ELF*) in relation to exudate lysozyme (*ELZ*) in RA and non-RA exudates

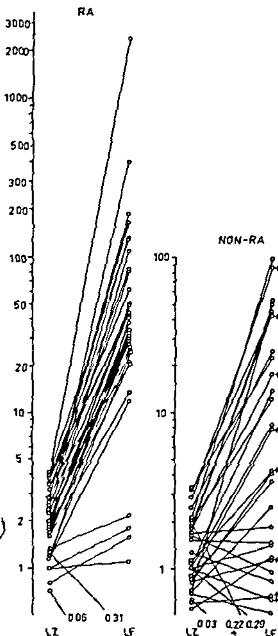


Fig 3 Exudate/plasma ratios for lactoferrin (*LF*) and lysozyme (*LZ*) in RA and non RA patients Lines are drawn between LZ and LF ratios in individual patients Note the logarithmic scale + = samples from patients with Reiter s syndrome

sampling as a calcium chelator Furthermore complete removal of cells from samples before freezing is of obvious importance It was found necessary to treat all samples with hyaluronidase to ascertain an even distribution of the cells within the sample prior to cell counting Furthermore the hyaluronidase treatment was necessary to permit complete separation of cells from the exudate before the analysis Such treatment has not been utilized in the studies cited above

The excess concentrations of leukocytic proteins in joint exudates clearly reflect local release of these components Since the neutrophil contents of LF and LZ are similar (10, 20) the preponderance of LF must be sought in a preferential release from cells or in a less rapid elimination of this protein from the exudate or both

The data of Bertino et al (4) suggest that granulocytes in joint effusions are destroyed in situ after a residence time similar to that in blood However the existence of a population of cells leaving the exudate more or less intact cannot be excluded Such cells may release part of their contents during residence in the exudate Leakage of neutrophil granule components during phagocytosis is a well established phenomenon, and such release can occur at dissimilar rates for different proteins (19)

With regard to elimination from the exudate Bennett et al (1) found the half life of LF in a joint to be as long as about 20 days However the data are not definitive since turnover was monitored by external counting of ^{59}Fe injected bound to LF Such measurements do not indicate whether the iron protein is located in exudate or synovial lining and in addition the iron may not have remained bound to LF throughout the measurement period In the rat van Snick et al (29) have demonstrated a rapid elimination of Fe LF from blood plasma by the reticuloendothelial system Accordingly reliable data on the clearance of LF from a joint effusion should be sought by measurement of labelled protein molecules in repeated exudate samples

The exudate LF concentrations as related to leukocyte counts were considerably higher in RA than in non RA exudates This may indicate that the extent of release of intraleukocytic components differs and that the role of injurious factors from neutrophils may be greater in RA than in other arthritides A good illustration of the importance of leukocyte mediated inflammation is the dramatic remission seen with drug induced neutropenia in active RA

It should be noted that neither LF nor LZ has any known tissue-destroying or irritant properties LF has no enzymatic activity LZ digests a polysaccharide linkage in bacterial cell wall proteoglycan but has no known substrate in the mammalian body It has recently been demonstrated by Greenwald (6) that LZ does not induce any changes

in cartilage proteoglycan It follows that LF and LZ are to be understood solely as markers of release of components from leukocytes LZ is distributed roughly equally between the two main cytoplasmic granule classes of the neutrophil the primary and secondary (specific) granules Of these only the primary granules are of a lysosomal nature in that they contain hydrolytic enzymes i a protease (30) LF occurs only in the secondary granules and LF levels may therefore not closely reflect the release of hydrolytic enzymes However LF is advantageous in being a neutrophil specific protein in contrast to other proteins used as neutrophil leukocyte markers

Serial determinations during the course of arthritides are needed to define further the importance of neutrophil components in various forms of arthritic tissue injury

ACKNOWLEDGEMENTS

This study was supported by the Swedish Medical Research Council (No 03X-4726) and by the Alfred Osterlund Foundation

REFERENCES

1 Bennett R M Eddie-Quartey A C & Holt P J L Lactoferrin—an iron binding protein in synovial fluid Arthritis Rheum 16 186 1973
2 Bennett R M & Mohla C A solid phase radioimmunoassay for the measurement of lactoferrin in human plasma variations with age sex and disease J Lab Clin Med 88 156 1976
3 Bennett R M & Skosey J L Lactoferrin levels in synovial fluid—its origin and significance as a marker of inflammation (Abstract) J Rheumatol (Suppl) 1 44 1974
4 Bertino J R Hollingsworth J W & Cashmore A R Granulocyte kinetics in rheumatoid effusions studied by a biochemical label Trans Assoc Am Physicians 76 63 1963
5 Decoteau E Yurchak A M Partridge R E H & Tomasi T B Lactoferrin in synovial fluid of patients with inflammatory arthritis Arthritis Rheum 15 324 1972
6 Greenwald R A Lack of effect of lysozymes on cartilage proteoglycans Arch Biochem Biophys 175 520 1976
7 Greenwald R A Josephson A S Diamond H S & Tsang A Lysozyme (LZ) as a parameter of cartilage degradation Arthritis Rheum 15 111 1972
8 Gullberg R Vitamin B_{12}-binding proteins in normal human blood plasma and serum Scand J Haematol 9 639 1972
9 — Vitamin B_{12}-binding proteins in blood plasma and synovial fluid in arthritis Scand J Rheumatol 1 129 1972
10 Hansen N E & Andersen V Lysozyme activity in human neutrophilic granulocytes Br J Haematol 24 613 1973
11 Hansen N E Malmquist J & Thorell J Plasma myeloperoxidase and lactoferrin measured by radioimmunoassay relations to neutrophil kinetics Acta Med Scand 198 437 1975
12 Harris E D & Krane S M Collagenases Specific involvement of collagenase in normal physiology and pathology N Engl J Med 291 652 1974
13 Hollander J L McCarty D J Astorga G & Castro-Murillo E Studies on the pathogenesis of rheumatoid joint inflammation I The R A cell and a working hypothesis Ann Intern Med 62 271 1965
14 Janoff A Mediators of tissue damage in human polymorphonuclear neutrophils Ser Haematol III I 96 1970
15 Janoff A Feinstein G Malemud C J & Elias J M *Degradation of cartilage proteoglycan by human* leukocyte granule neutral proteases—a model of joint injury I Penetration of enzyme into rabbit articular cartilage and release of $^{35}SO_4$ labeled material from the tissue J Clin Invest 57 615 1976
16 Johansson B G & Malmquist J Quantitative immunochemical determination of lysozyme (muramidase) in serum and urine Scand J Clin Lab Invest 27 255 1971
17 Josephson A S & Greenwald R A Lysozyme as a component of human cartilage In Lysozyme (ed E F Osserman R E Canfield & S Beychok) p 411 Academic Press New York 1974
18 Keiser H Greenwald R A Feinstein G & Janoff A Degradation of cartilage proteoglycan by human leukocyte granule neutral proteases—a model of joint injury II Degradation of isolated bovine nasal cartilage proteoglycan J Clin Invest 57 625 1976
19 Leffell M S & Spitznagel J K Intracellular and extracellular degranulation of human polymorphonuclear azurophil and specific granules induced by immune complexes Infect Immun 10 1241 1974
20 Masson P L Heremans J F & Schonne E Lactoferrin an iron binding protein in neutrophilic leukocytes J Exp Med 130 643 1969
21 Oka M & Seppäla O Muramidase (lysozyme) in joint fluid and serum of rheumatic patients Acta Rheumatol Scand 16 223 1970
22 Oronsky A Ignarro L & Perper R Release of cartilage mucopolysaccharide-degrading neutral protease from human leukocytes J Exp Med 138 461 1973
23 Pruzanski W & Ogryzlo M A Lysozyme in rheumatic diseases Semin Arthritis Rheum 1 361 1972
24 Pruzanski W Ogryzlo M A & Katz A Lysozyme production and abnormalities in rheumatic diseases In Lysozyme (ed E F Osserman R E Canfield & S Beychok) p 419 Academic Press New York 1974
25 Pruzanski W Saito S & Ogryzlo M A The

significance of lysozyme (muramidase) in rheumatoid arthritis I Levels in serum and synovial fluid Arthritis Rheum 13 389 1970
26 Pryce Jones R H Saklatvala J & Wood G C Neutral protease from the polymorphonuclear leucocytes of human rheumatoid synovial fluid Clin Sci Mol Med 47 403 1974
27 Ropes M V Bennet G A Cobb S Jacox R & Jesser R A 1958 revision of diagnostic criteria for rheumatoid arthritis Bull Rheum Dis 9 213 1958
28 Rumke P Visser D Kwa H G & Hart A A M Radio-immuno assay of lactoferrin in blood plasma of breast cancer patients lactating and normal women prevention of false high levels caused by leakage from neutrophile leucocytes in vitro Folia Med Neerl 14 156 1971
29 van Snick J L Masson P L & Heremans J F The involvement of lactoferrin in the hyposideremia of acute inflammation J Exp Med 140 1068 1974
30 Spitznagel J K Dalldorf F G Leffell M S Folds J D Welsh I R H Cooney M H & Martin L E Character of azurophil and specific granules purified from human polymorphonuclear leukocytes Lab Invest 30 774 1974
31 Weissman G Lysosomal mechanisms of tissue injury in arthritis N Engl J Med 286 141 1972
32 Weissmann G Zurier R B & Hoffstein S Leukocytic proteases and the immunologic release of lysosomal enzymes Am J Pathol 68 539 1972
33 Wooley D E Lindberg K A Glanville R W & Evanson J M Action of rheumatoid synovial collagenase on cartilage collagen Different susceptibilities of cartilage and tendon collagen to collagenase attack Eur J Biochem 50 437 1975

Acta Med Scand 202 319–322 1977

The Value of Thermography and the Determination of Fibrin–Fibrinogen Degradation Products in the Diagnosis of Deep Venous Thrombosis

L G Bystrom T Larsson L Lundell and P E Åbom

From the Departments of Diagnostic Radiology Internal Medicine and Surgery Central Hospital Jonkoping Sweden

ABSTRACT Fifty one patients with leg symptoms indicating deep venous thrombosis (DVT) were investigated concomitantly with thermography and phlebography Altogether 26 legs with phlebographically proven DVT exhibited a thermographic picture typical of DVT So however, did 3 out of 25 legs with no DVT A 94% agreement was found between phlebography and thermography when applied to legs with suspect DVT The serum levels of fibrin–fibrinogen degradation products (FDP) were assayed in 58 patients attending hospital for suspect DVT These patients were free from other diseases known to be followed by raised FDP levels With borderline significance, the DVT group had higher FDP values The usefulness of this laboratory test in the diagnosis of DVT is, however questioned

It has become evident during the last decade that the clinical diagnosis of deep venous thrombosis (DVT) seldom surpasses 50% accuracy (2 12 13) Consequently objective diagnostic examinations are required and the one used most frequently is ascending phlebography Although the diagnostic accuracy is satisfactory phlebography has certain disadvantages for the patient as well as for the doctor (1 11) In view of these circumstances more convenient diagnostic procedures have been evolved the most commonly applied being the ^{125}I fibrinogen uptake test the Doppler sound technique and plethysmography (19) Thermography has recently been introduced as a safe and simple method for diagnosing DVT (3 6)

Chemical laboratory tests have also been introduced the results of which have been claimed to be valuable for identification of patients with either incipient or established DVT (16)

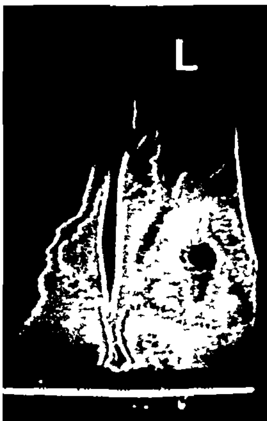
The aim of the present study was to evaluate the diagnostic precision of the non invasive technique of thermography and the assay of fibrin–fibrinogen degradation products (FDP) in patients attending hospital for symptoms indicating DVT

PATIENTS AND METHODS

Phlebography The phlebographic investigation was carried out according to the principles outlined by Greitz (9) Nylander (21) Rabinov and Paulin (23) The contrast medium (Isopaque® Cerebral Nyegaard) was injected into a distal foot vein In order to direct the contrast medium into the deep venous system a tourniquet was applied around the ankle The examination table was tilted 60° and both frontal and side view films were taken of the calf and thigh The phlebographic criteria of DVT were the same as those given in the literature (17 21) The radiographs were interpreted by a radiologist who was unaware of the results of the other investigations The phlebographic examination was always performed after thermography the interval between the examinations ranging from 2 to 48 hours

Thermography The thermographic system used was a Bofors IR-camera (now manufactured by Philips) The heat irradiation from the patient was focused via a special mirror system on an indium antimonid detector which was cooled by liquid nitrogen The electric signal thus produced was amplified and transmitted to a display unit where the thermogram was presented on a television screen The thermograms were recorded with a polaroid colour camera (Fig 1) The patients were examined in a special room with a temperature of 20–22 C To avoid venous pooling the patients legs were elevated 15° and about 15 min was allowed for thermal equilibrium between the skin and the ambient temperature Immediately before recording of the thermograms the legs were cooled with ethanol aerosol The fronts of the calves and thighs were studied together with the posterior view of the calves and popliteal fossa

significance of lysozyme (muramidase) in rheumatoid arthritis I Levels in serum and synovial fluid Arthritis Rheum 13 389 1970
26 Pryce Jones R H Saklatvala J & Wood G C Neutral protease from the polymorphonuclear leucocytes of human rheumatoid synovial fluid Clin Sci Mol Med 47 403 1974
27 Ropes M V Bennet G A Cobb S Jacox R & Jesser R A 1958 revision of diagnostic criteria for rheumatoid arthritis Bull Rheum Dis 9 213 1958
28 Rumke P Visser D Kwa H G & Hart A A M Radio-immuno assay of lactoferrin in blood plasma of breast cancer patients lactating and normal women prevention of false high levels caused by leakage from neutrophile leucocytes in vitro Folia Med Neerl 14 156 1971
29 van Snick J L Masson P L & Heremans J F The involvement of lactoferrin in the hyposideremia of acute inflammation J Exp Med 140 1068 1974
30 Spitznagel J K Dalldorf F G Leffell M S Folds J D Welsh I R H Cooney M H & Martin I E Character of azurophil and specific granules purified from human polymorphonuclear leukocytes Lab Invest 30 774 1974
31 Weissman G Lysosomal mechanisms of tissue injury in arthritis N Engl J Med 286 141 1972
32 Weissmann G Zurier R B & Hoffstein S Leukocytic proteases and the immunologic release of lysosomal enzymes Am J Pathol 68 539 1972
33 Wooley D E Lindberg K A Glanville R W & Evanson J M Action of rheumatoid synovial collagenase on cartilage collagen Different susceptibilities of cartilage and tendon collagen to collagenase attack Eur J Biochem 50 437 1975



3 Thermogram showing a normal thigh and one with T (*L*)

extensiveness of the thrombus However upon a statistical analysis (χ^2 test) of the entire material the DVT group had significantly higher amounts of FDP than the group without DVT ($p<0.05$)

DISCUSSION

Our results demonstrate that thermography is almost as accurate as phlebography in detecting DVT Our experience completely agrees with that of other investigators (3 6) Thermography is preferable to phlebography in that it is non invasive convenient for the patient rather rapid easily repeated at short intervals and without risks for the patient (1 18) It should however be noted that thermography cannot replace phlebography when surgical or thrombolytic therapy is planned The mechanism behind the increased heat emission from legs with DVT has been suggested to be an inflammatory reaction around the thrombus and/or increased skin blood flow (6) An accelerated skin blood flow has been demonstrated both with phlebographic (22) and with plethysmographic techniques (14)

Widespread inflammatory reactions give rise to thermographically false positive results but we have hitherto had no false negative ones Muscle hematoma which often presents symptoms indicating DVT seems not to be followed by enhanced heat irradiation Two of our patients developed clinical signs of hematoma but their thermograms indicated no DVT A localized inflammatory reaction such as a thrombophlebitis produces a local hot area corresponding to the finding at the clinical

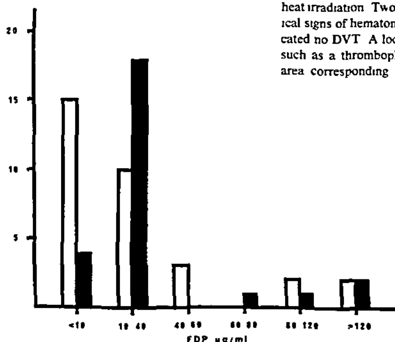


Fig 4 Distribution of FDP values in patients with (■) or without DVT (□)



Fig 1 The thermographic system in use

The study was made on 51 consecutive patients attending hospital with suspect symptoms of DVT in a leg There were 24 men mean age 63 3 years (range 32-83) and 27 women mean age 57 7 years (range 21-84)

The thermographic appearance of a normal calf is presented in Fig 2 The calf muscles produce some heat but a characteristic cool area is seen pretibially Fig 3 shows a normal thigh with a cool area over the tendon apparatus and the patella When a calf thrombus is present there is a diffuse enhanced heat emission and the pretibial cooling is absent (Fig 2) This diffuse heat irradiation is also seen on the posterior view when a DVT is present When the thrombus grows into the femoral vein the prepatellar cooling is diminished or absent and the heat emitted from the thigh is enhanced (Fig 3)

FDP assay The assay of FDP has been in routine use in our hospital since 1974 Patients with concomitant diseases known to be followed by elevated concentration of FDP in serum (15) were excluded from the study

The study was made on 58 patients all examined with ascending phlebography There were 28 men mean age 60 0 years (range 18-83) and 30 women mean age 54 3 years (range 21-84) Twenty three of these patients were not investigated with thermography since this technique was not introduced in our hospital until Sept 1975

On the day the patient attended hospital serum was withdrawn for estimation of FDP We have used a rapid slide test in which the latex reagent is sensitized with anti FDP antibodies (Thrombo Wellco test Borroughs Wellcome) This assay primarily allows a semiquantitative measurement of the fibrinolysis end products fragments D and E (7)

RESULTS

Thermography There was a phlebographically proven DVT in 26 legs At thermography all these legs presented a heat pattern typical of DVT Among the 26 thrombi only 4 were restricted to the calf the other 22 also occupied the femoral vein In one of these 22 legs, the thermoexamination indicated a thrombus only in the calf veins Otherwise complete agreement existed between thermography and phlebography regarding the extensiveness of the thrombus



Fig 2 Thermogram showing a normal calf (L) and one with DVT

A deep venous system free from thrombosis was found at phlebography in 25 legs The thermographic appearance of three of these legs indicated DVT These legs were all affected by widespread inflammatory reactions of various origin The overall agreement between phlebography and thermography in the present study was 94%

FDP assay The phlebographic examination revealed a DVT in 26 patients 85% of whom had FDP in a concentration of 10-40 μg/ml or higher (Fig 4) The majority (69%) of patients with DVT had 10-40 μg/ml FDP in serum Thirty two patients had no DVT but only 47% of them had FDP concentrations of less than 10 μg/ml A remarkable finding was that 31% of patients with no DVT had 10-40 μg/ml FDP in serum

We were unable to demonstrate any association between the amounts of FDP in serum and the

Acta Med Scand 202 323–326 1977

Electro-Retinal Abnormalities in Heterozygotes of Renal-Retinal Dysplasia

Study of Two Families with Medullary Cystic Disease and Retinitis Pigmentosa

Barend L Hogewind Jan J Veltkamp Bettine C P Polak and Leendert A van Es

From the Nephrology Division of the Department of Medicine and the Department of Ophthalmology University Hospital Leiden The Netherlands

ABSTRACT The relatives of two patients with medullary cystic disease associated with retinitis pigmentosa were studied A new case was found in one of these families and consanguinity of the parents was established in another Conventional fundoscopic examination of relatives without renal disease did not show retinal abnormalities but electro ophthalmologic investigation demonstrated retinal dysfunction in three relatives including two of the four parents who may be considered obligatory heterozygotes under the assumption of autosomal recessive inheritance of this syndrome Less severe electro-ophthalmological abnormalities were observed in the other two parents It is considered highly probable that all three patients are homozygous for a mutant gene causing both the renal and the retinal abnormalities The results of this study support the view that medullary cystic disease associated with retinitis pigmentosa is transmitted as an autosomal recessive trait in contrast to the dominant form, which is reported not to be associated with eye abnormalities With respect to genetic counseling and donation of kidneys by relatives it is important to establish the mode of inheritance of cystic medullary disease in a given family Electro-ophthalmologic examination should therefore be included in the examination of families in which medullary cystic disease occurs

Hereditary renal disease is responsible for 10–20% of the cases of renal insufficiency necessitating kidney transplantation In adults polycystic disease of the kidney is the most frequent hereditary cause of chronic renal failure (10) In children medullary cystic disease and hereditary chronic nephritis (Alport s syndrome) are mainly responsible (3) Medullary cystic disease may be associated with retinitis pigmentosa Some authors prefer to define this association as a separate syndrome called hereditary renal retinal dysplasia (1 13) Renal retinal dysplasia consistently shows a recessive pattern of inheritance which is not always the case with medullary cystic disease without retinal involvement (1 9 11–15)

In our center 10 out of 250 transplant patients were known to have medullary cystic disease Two of these 10 patients were found to have retinitis pigmentosa and their families were investigated for renal and retinal abnormalities

Requests for reprints to B L Hogewind Nephrology Division University Hospital Bldg 30 Leiden The Netherlands

CASE REPORTS

Case 1

This man born in 1959 is the proband of family A (II 8) The pedigree of this family is shown in Fig 1 He was first seen by a pediatrician at the age of 5 because of polyuria polydipsia and enuresis The results of urinalysis were normal serum creatinine concentration 0 5 mg/100 ml BP 110/75 mmHg and the specific gravity of the urine 1 004 after a 12 hour period of dehydration In the following years his renal function deteriorated At the age of 11 the serum creatinine concentration was 5 7 mg/100 ml One kidney was removed when he received a cadaveric renal allograft at the age of 16 Histological investigation of the excised kidney showed medullary cystic disease Routine fundoscopy revealed retinitis pigmentosa

Table 1 *Results of the ophthalmological studies*
EOG=electro-ophthalmography ERG=electroretinography N=normal

Pedigree no	Age (y)	Vision	Visual fields	Color vision	Retinitis pigmentosa	EOG	ERG
Family A							
I 1	57	1 0	N	N	Absent	N	Borderline
I 2	54	1 25	N	N	Absent	Decreased	Decreased
II 6	19	1 0	N	N	Absent	N	Borderline
II 8	16	1 0	Central rest	Tritanomaly	Present	Decreased	Decreased
II 9	14	1 25	N	N	Absent	Decreased	Decreased
II 10	12	1 0	N	N	Early signs	N	Decreased
Family B							
III 1	52	1 0	N	N	Absent	N	Decreased
III 3	48	1 0	N	Protanomaly	Absent	Borderline	N
IV 1	17	1 0	N	N	Absent	N	N
IV 2	16	0 3	Central rest	Tritanomaly	Present	Decreased	Decreased

Case 2

This man born in 1959 is the proband of family B (IV 2) His pedigree is shown in Fig 2 At the age of 8 years when he was seen by his family doctor because of pallor and anorexia it was found that polydipsia polyuria and nycturia had been present for several years The serum creatinine concentration was elevated (1 7 mg/100 ml) and the specific gravity of the urine was low The results of urinalysis were normal Hb concentration was 9-8 g/100 ml and BP normal (125/95 mmHg) I v pyelography and miction cystography did not show abnormalities On the basis of a renal biopsy specimen the diagnosis of interstitial nephritis was made Progressive azotemia developed necessitating dialysis at the age of 13 At the age of 14 his kidneys were removed and he was given a cadaveric kidney Macroscopically the kidneys showed medullary cystic disease Subsequent fundoscopy revealed retinitis pigmentosa

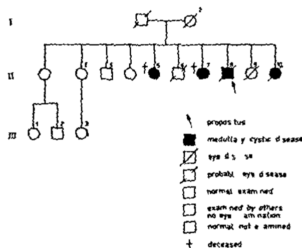


Fig 1 Pedigree of family A

FAMILY INVESTIGATION

Family A

Two older sisters of the proband died at home of terminal renal failure at the ages of 11 (1964) and 12 (1970) (Fig 1) Both suffered from polydipsia and polyuria and later developed anemia and azotemia Their eyes were not examined and autopsies were not performed A younger sister (II 10), born in 1963 appeared to have had polydipsia and polyuria for some time without much attention being paid to

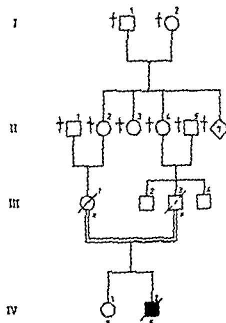


Fig 2 Pedigree of family B Symbols as in Fig 1

these symptoms She had a creatinine clearance of 50 ml/min Maximal specific gravity of the urine was 1 008 Both fundoscopy and electro-ophthalmological investigation showed abnormalities indicating retinitis pigmentosa Nine other relatives had normal kidney function including a normal concentrating ability

The results of ophthalmological examination of these relatives are shown in Table I The fundi showed no abnormalities in routine fundoscopy Except in the proband (II 8) neither color vision nor the fields of vision were restricted However EOG gave significantly abnormal results in two relatives (I 2 and II 9) In two other relatives (I 1 and II 6) the findings were dubious As far as could be ascertained the parents are not related No other cases of early death or renal or ophthalmological disease could be found in this family

Family B

The parents of the proband are related Diseases of kidneys or eyes were not known to exist in this family and early death was not reported Both parents and the proband s sister have normal renal function including normal urinary concentration The mother (III 1) had a distinctly abnormal electroretinogram (ERG) (Table I) the father (III 3) a borderline EOG The color vision defect (protanomaly) found in the father is thought to be a coincidence In the sister (IV 1) the results of the eye examination were normal

COMMENTS

Cystic disease of the renal medulla was first described by Smith and Graham (16) in 1945 as a slowly progressive kidney failure characterized by early impairment of the kidney s capacity to concentrate urine resulting in polyuria nycturia polydipsia and frequently elevated sodium excretion Rather typical is the absence of proteinuria and abnormalities in the urinary sediment Without hemodialysis or kidney transplantation the disease is lethal in the 2nd or 3rd decade The disease is equally frequent in females and males and has been reported to occur both sporadically as well as to run in families

Early onset of the disease (at an average age of 10 5 years) has been associated with homozygosity for a recessive trait and late onset (at an average age of 26 7 years) with heterozygosity for a dominant character (5) However Giangiacomo et al (6) who reported a kindred with presumptive dominant inheritance of the early onset form (10 1 years) advocate re evaluation of the differentiation of cystic medullary disease on the basis of the age at onset in association with the mode of inheritance These authors also recommend the examination of each member of a family in which medullary cystic disease is found in order to collect more information about the mode of inheritance

Another contribution to the discussion on the mode of inheritance of medullary cystic disease was made by Strauss (18) who concluded that the combination of this disease and retinitis pigmentosa only occurs in kindreds with recessive inheritance Furthermore retinitis pigmentosa was not encountered in 18 sporadic cases (17) or in 30 cases with well established dominant inheritance (7) It is highly probable that both the renal and the retinal disorders are caused by homozygosity for a single pleiotropic gene since all individuals with completely developed retinitis pigmentosa found in such families also suffered from the kidney condition (1 9 11–15) Several authors (1 13) therefore propose that the syndrome for which only a recessive mode of inheritance has been observed so far should be given a name of its own renal retinal dysplasia

The five patients described in the present report all show the clinical picture of early onset medullary cystic disease Anemia due to kidney failure was the main reason for consulting a physician Symptoms of polyuria and polydipsia were present rather early Except for hyposthenuria even after dehydration urinalysis invariably gave normal results Hypertension does not appear to be an important feature of this syndrome Four of the five patients were in the terminal stage between their 11th and 13th year The histology of the kidneys removed before transplantation in both probands showed the typical picture of medullary cystic disease The medulla showed extensive destruction due to numerous cysts Microscopically periglomerular and peritubular fibrosis was seen together with hyalinized glomeruli and dilated tubules i e changes indistinguishable from interstitial nephritis

These two probands showed retinitis pigmentosa which implies that recessive inheritance was at play The arguments in favour of recessive inheritance are indeed strong There is no vertical transmission of the disease In family B there is consan

guinity between the parents The completely developed picture of retinitis pigmentosa was only observed in the three living patients with kidney involvement These three individuals and the two who died are in our opinion to be considered as homozygotes for the renal retinal dysplasia gene

The electro-ophthalmological abnormalities found in relatives were demonstrated by EOG and ERG i e techniques which measure the functional integrity of the photoreceptors and the pigment epithelium and are therefore used for the early detection of retinitis pigmentosa and the demonstration of carriership for a variety of hereditary eye diseases (4 8) Especially the report by Berson and Kanters (2) on the ERG abnormalities in carriers of recessive retinitis pigmentosa supports the hypothesis that these relatives (A I 2 A II 9 and B II I) carry the gene for renal retinal dysplasia In this respect the EOG seems to have less significance although the number of subjects investigated is rather small It is of interest to note that the ERG was abnormal in the girl (II 10) of family A at the age of 12 when only early signs and symptoms of kidney disease were present

REFERENCES

1 Arasthi P S Erickson D G & Gardner K D Hereditary renal retinal dysplasia and the medullary cystic disease–nephronophthisis complex Ann Intern Med 84 157 1976
2 Berson E L & Kanters L Cone and rod responses in a family with recessively inherited retinitis pigmentosa Arch Ophthalmol 84 288 1970
3 Betts P R & Forrest Hay J Juvenile nephronophthisis Lancet 2 475 1973
4 François J Chorioretinal heredo-degeneration Proc Roy Soc Med 54 1109 1961
5 Gardner K D Evolution of clinical signs in adult onset cystic disease of the renal medulla Ann Intern Med 74 47 1971
6 Giangiacomo J Monteleone P L & Witzleben C L Medullary cystic disease vs nephronophthisis JAMA 232 629 1975
7 Goldman S H Walker S R Merigan T C et al Hereditary occurrence of cystic disease of renal medulla N Engl J Med 274 984 1966
8 De Haas E B H Van Lith G H M Rijnders J et al Usher s syndrome Doc Ophthalmol 28 166 1970
9 Herdman R C Good R A & Vernier R L Medullary cystic disease in two siblings Am J Med 43 335 1967
10 Lowrie E G Lazarus J M Mocelin A F et al Survival of patients undergoing chronic hemodialysis and renal transplantation N Engl J Med 288 863 1973
11 Mainzer F Saldino R M Ozonoff M B et al Familial nephropathy associated with retinitis pigmentosa cerebellar ataxia and skeletal abnormalities Am J Med 49 556 1970
12 Meier D A & Hess J W Familial nephropathy with retinitis pigmentosa A new oculorenal syndrome in adults Am J Med 39 58 1965
13 Schimke R N Hereditary renal retinal dysplasia Ann Intern Med 70 735 1969
14 Senior B Familial renal retinal dystrophy Am J Dis Child 125 442 1973
15 Senior B Friedman A I & Braudo J L Juvenile familial nephropathy with tapetoretinal degeneration A new oculorenal dystrophy Am J Ophthalmol 52 625 1961
16 Smith C H & Graham J B Congenital medullary cysts of kidneys with severe refractory anemia Am J Dis Child 69 369 1975
17 Strauss M B Clinical and pathological aspects of cystic disease of renal medulla analysis of eighteen cases Ann Intern Med 57 373 1962
18 — Microcystic disease of the renal medulla In Diseases of the kidney (ed M B Strauss and L G Welt) p 1264 Little Brown & Co Boston 1971

Acta Med Scand 202 327–329 1977

Fluid Turnover in Renal Cysts

L Jacobsson B Lindqvist G Michaelson and P Bjerle

From the Departments of Clinical Chemistry Medicine and Clinical Physiology University of Umea Umea Sweden

ABSTRACT Cystic puncture was performed percutaneously in 18 patients with solitary renal cysts and in 22 with multiple, congenital renal cysts With the aid of tritiated water it was possible to estimate the fluid turnover in the cysts and compare it with their volume, pressure and potassium and creatinine levels Fluid turnover was rapid in all the renal cysts Two to five hours after i v injection of tritium, the tritium concentration in cystic fluid averaged 88% of the concentration in plasma fluid in patients with polycystic kidneys and 73% in patients with solitary cysts Fluid turnover was more rapid in small than in large cysts but there was no such difference between cysts with high and low pressure It is possible that the fluid turnover was slightly faster in cysts with high potassium and creatinine levels than in those with low levels The results show that the fluid turnover in a renal cyst of 10 ml is considerable—probably more than 100 ml/24 hours This indicates that fluid inflow to the cyst comes mainly from cells in the cyst wall and not from a single glomerule Fluid probably leaves the cyst actively via cells in the cyst wall, since the fluid turnover does not increase with high cyst pressure The fluid turnover is probably secondary to the active solute transportation which is performed by the cyst cells This means that these cells have a tubular cell like function and should respond to pharmacotherapy

Research in the pathophysiology of renal cysts is incomplete The cysts contain fluid which is regularly renewed This is easily verified by injecting roentgen contrast fluid into a renal cyst the contrast grows steadily fainter and disappears completely within a period ranging from a few hours to days It is not clear which laws govern the flow of fluid into and out of the cyst The volume pressure and most important the rate of growth of the cyst presumably depend on the balance between this inflow and outflow

As is well known multiple cysts in one and the same patient differ in size and their rate of growth varies between individuals The concentrations in the cystic fluid of electrolytes creatinine and the total number of osmotically active substances vary considerably between cysts in patients with congenital multiple cysts (3 4 5) The turnover of cystic fluid likewise varies between cysts (3) Bricker and Patton (3) found that inulin and PAH injected intravenously could be demonstrated in fluid from some of the cysts which were punctured 8–22 min after the injection though in most of the punctured cysts they could not detect any inulin or PAH (or only very small amounts) They concluded that some cysts seem to be connected with active nephrons Cyst pressure varies considerably in all patients with polycystic kidneys (2) We showed in 1971 (1) that the pressure in renal cysts varies between 6 and 70 mmHg with an average of 25 mmHg for multiple congenital cysts and 19 mmHg for single cysts Cyst pressure increased with increasing uremia

The size pressure rate of growth fluid turnover and solute concentration can thus vary greatly in one and the same person We have studied the rate of fluid turnover in renal cysts and compared it with the size pressure and solute concentration of the cysts to study whether these factors influence one another This report concludes with a discussion of various pathophysiological mechanisms for cyst growth

PATIENTS AND METHODS

Twenty nine cysts in 18 patients with one or two renal cysts and 155 cysts in 22 patients with multiple congenital cysts were punctured percutaneously under TV fluoroscopy (1) Twenty five of the patients were women and 15 men mean age 51 years (range 32–77) The

creatinine level was normal or nearly normal in 22 patients between 170 and 850 μmol/l in 13 and higher than 850 μmol/l in 5

Fluid turnover in the cysts was measured with the aid of tritiated water which was injected i v 2–5 hours before cystic puncture The punctures took 5–30 min to perform depending on the number of cysts punctured The number of counts per ml fluid was determined in cystic fluid and in blood water respectively In patients with polycystic kidneys a sufficient quantity of fluid for tritium determination was extracted from 4 cysts on an average (range 2–9) In 2 patients with single renal cysts the cyst was punctured twice the second puncture being performed about one hour after the first The blood sample was taken 5–15 min after the final cyst had been punctured The percental number of counts per ml in cystic fluid in relation to serum was calculated The number of counts per ml of blood water is nearly constant 2–5 hours after the injection of tritiated water The tritium determination was performed with a scintillation counter type Intertechnique ABAC 40

Cyst volume was estimated from the amount of fluid which was possible to extract by means of puncture However since the position of the needle varied this estimation is only approximate Volume was estimated in about half the cases

Cyst pressure was measured according to a method described previously (1) Pressure was recorded in the majority of patients with multiple cysts but only in a few of those with single cysts

The potassium and creatinine levels in cystic fluid were determined in 25 cysts from which a sufficient quantity of fluid could be extracted

RESULTS

In patients with polycystic kidneys the tritium concentration of cystic fluid averaged 88% (range 29–106) of that of serum 2–5 hours after the i v injection of tritium In single cysts the average value was 73% (range 9–98) The fluid turnover thus varied greatly However after 2–5 hours the tritium concentration of cystic fluid had reached at least half the concentration in blood in 97% of the cysts in polycystic patients and in 70% of those in patients with single cysts Fluid turnover was thus rapid in nearly all cysts

The tritium concentration of cystic fluid in relation to the level in serum of patients with polycystic kidneys was on an average 82 5% in the first cyst punctured and 81 3% in the last This shows that after 2–5 hours the average tritium concentration of cystic fluid had not reached the same value as in blood but that the first rapid phase of equalization had given way to its slow phase at this point in polycystic kidneys

In polycystic patients the cyst volumes exceeded 10 ml in 22 cysts and fell below 10 ml in 74 The mean values for tritium concentration of cystic fluid in relation to the level in serum were 82 and 90% respectively Four single cysts exceeded 100 ml in volume and 9 were below 100 ml The mean values for tritium concentration were 48 and 72% respectively The results show that the average fluid turnover is more rapid in small cysts than in large

Two single cysts were punctured twice after 150 and 215 min and after 135 and 200 min respectively The tritium concentration of cystic fluid was 58 and 74% of that in blood in the former case 26 and 41% in the latter The tritium activity in the same amount of blood plasma water remained constant at 32400 and 22600 counts per min respectively throughout the investigation The amount of fluid withdrawn was 8 and 10 ml at the first punctures 25 and 55 ml at the second

We were not able to calculate the precise turnover from the figures above The difficulties lie in the fact that a certain amount of fluid is withdrawn from the cyst for the estimation of tritium activity and we do not know whether this amount is replaced in the cyst Neither do we know the cyst s exact volume The cyst is drained of fluid at the second puncture but possibly not completely There is also the problem of leakage after a puncture Furthermore the mean tritium concentration of the outflow is uncertain Instead the turnover of cystic fluid has been estimated using the following formula

$$V_{in}\times T_s - V_{out}\times T_{out} = V_2\times T_2 - V_1\times T_1$$

(V_{in}=volume of inflow between the two punctures V_{out}=volume of outflow between the two punctures V_2=volume of cyst at the second puncture V_1=volume of cyst after the first puncture T_s=tritium concentration of serum water T_{out}=mean tritium concentration of outflow between the two punctures T_2=tritium concentration in cystic fluid at the second puncture T_1=tritium concentration at the first puncture)

The precise turnover must lie between the theoretical maximal and minimal values V_{in} is minimal when V_{out} is zero during the time of investigation (65 min) and the cyst is refilled after the puncture V_{in} is maximal when V_{out} is the same as V_{in} and T_{out} is close to T_2 Our results indicate that the fluid turnover in the first single cyst was 8–29 and in the second 4–8 times per 24 hours The fluid turnover

n a single renal cyst can probably exceed 200 ml/ 24 hours

In patients with polycystic kidneys the pressure n 42 cysts was higher than 20 mmHg and in 51 ysts it was lower than 20 mmHg The mean values or the concentration of tritium in cystic fluid in relation to the level in serum were 81% (range 36–100) and 87% (range 37–104) respectively The regression of tritium concentration on the pressure s probably significant (0 05>p>0 01) We were thus unable to find that cysts with high pressure have a more rapid fluid turnover than cysts with low pressure

In patients with polycystic kidneys there were 5 cysts with a potassium level in cystic fluid of 10 mmol/l or more and 20 cysts with less than 10 mmol/l The tritium contents averaged 99 and 85% respectively (p<0 01) It is possible that cysts with a high potassium concentration have a faster fluid turnover but the material was too small for definite statistical proof

In patients with polycystic kidneys there were 10 cysts with a creatinine level in cystic fluid of more than 250 μmol/l and 8 cysts with less than 250 μmol/l The mean tritium levels in cystic fluid were 86 and 82% respectively of the tritium level in plasma fluid with a wide distribution

DISCUSSION

All cysts have fluid turnover in many cases rapid This and the fact that the fluid in many cysts is hypertonic indicates that the cyst is an extended part of the nephron The flow through a nephron that would achieve a balance between blood and cystic fluid in 2 hours must be considerable Our results with the two single cysts indicate a fluid turnover of about 6–20 times in 24 hours Owing to the complexity of such calculations these findings are no more than estimates In adults a cyst is connected to a single glomerulus (5 6 7) It is unlikely that one nephron filling a cyst with a volume of 10 ml could achieve as great a urinary flow as 60–200 ml/24 hours Fluid inflow to the cyst therefore probably occurs mainly in the form of diffusion from the tubular cells of the cyst wall

Similar reasoning can be applied to the outflow from a cyst This cannot take place via a single nephron tubule it must occur via the cells and the lymph of the cyst wall The mechanism may be purely mechanical at a high pressure or perhaps it is brought about by cells which actively absorb fluid by means of active solute transportation and passive fluid diffusion which is independent of hydrostatic pressure We were not able to show that cysts with high pressure have a faster fluid turnover than those with low pressure This indicates that fluid is actively absorbed and the lymph flow is probably not therefore of major significance

The cystic fluid turnover rate should be maximal if the cells in the wall have a great capacity for both diffusing and absorbing fluid the cyst pressure will then be moderate and the cysts in all likelihood relatively small If the cells in the cyst walls are essentially similar to those in distal tubules the fluid absorbing capacity should be great and the fluid inflow small while the cyst pressure should be low and the fluid turnover slow If the cells in the cyst walls on the other hand are essentially from proximal tubules both the fluid absorbing and the fluid inflow capacity should be great the cyst pressure could be high and the fluid should increase We have however been unable to find any differences in pressure between large and small cysts in one and the same patient (1)

The possibilities of influencing the growth of cysts medicinally may depend on whether a medicine can be found which will reduce the function of proximal tubules and increase that of distal tubules We have so far tested acetazolamide (2) chlorpropamide and arginine vasopressin (unpublished data) without any clearly favourable results Polycystic kidneys shrink after renal transplantation

REFERENCES

1 Bjerle P Lindqvist B & Michaelson G Pressure measurements in renal cysts Scand J Clin Lab Invest 27 135 1971
2 Bjerle P Lindqvist B Michaelson G Ringqvist I & Rudolphi O The effect of acetazolamide on cystic pressure and renal function in polycystic renal disease IRCS (73 5) 8 5 2 1973
3 Bricker N S & Patton J F Cystic disease of the kidneys Am J Med 18 207 1955
4 Gardner K D Composition of fluid in twelve cysts of a polycystic kidney N Engl J Med 281 985 1969
5 Lambert P P Polycystic disease of the kidney Arch Pathol 44 34 1947
6 Osathanond V & Potter E Pathogenesis of polycystic kidneys Arch Pathol 77 485 1964
7 Potter E L Normal and abnormal development of the kidney Year Book Medical Publishers Chicago 1972

Acta Med Scand 202 331–334 1977

Deleterious Effects of Cardiac Pacing in a Patient with Mitral Insufficiency

O Edhag B Fagrell and H Lagergren

From the Departments of Medicine and Surgery Serafimerlasarettet Stockholm Sweden

ABSTRACT A 70 year-old, artificially paced woman with dizziness and extremely low physical capacity exhibited a systolic BP varying from one moment to another in standing position it was not measurable With the aid of a strain gauge technique the amplitude of the pulse wave of her left thumb was recorded and shown to vary widely The variations were correlated to synchrony or asynchrony between atrial and ventricular activity Pronounced decreases in stroke volume and peripheral pulse volume were recorded with pacemaker induced beats compared with idioventricular beats With artificial stimulation at a rate of 45/min thus avoiding competition but still protecting her from syncopes, she was free from symptoms

In most patients with serious cardiac conduction disturbances the implantation of an artificial pace maker will improve the cardiac haemodynamics considerably In some patients however the bene fit on cardiac output will be poor and in a few cases even deleterious It is of great importance to clarify the reasons for these negative results and if pos sible to change the mode of stimulation or other wise improve the situation A few earlier reports have dealt with the deleterious effects of pacemaker treatment in patients with certain types of cardiac disease (3 6)

The markedly negative effects of artificial cardiac pacing in a patient with multiple arrhythmias are presented in this report

CASE REPORT

The patient was a 70-year-old woman who had suffered from a sore throat complicated by inflammation of certain joints at 25 years of age A cardiac murmur was dis covered after this illness

In 1967 the patient had a syncopal attack followed some months later by sudden precordial chest pain An atrial fibrillation but no objective signs of a myocardial infarction was recorded In Jan 1970 she had a new attack of central chest pain which brought her into hos pital During the first few days after admission artificial cardiac pacing had to be introduced because of complete heart block and progressive cardiac failure The patient recovered somewhat as a result of this procedure but displayed a large number of multiple arrhythmias A myo cardial infarction of the diaphragmatic and lateral wall was recorded She was discharged in fairly good condi tion after 4 weeks During the following year she had several attacks of syncope which led to the implantation of a permanent R wave synchronous pacemaker This measure kept her in quite good condition for the next two years In 1972 however episodes of atrial flutter and fibrillation started and during the following 2 years she suffered from approximately 10 such attacks

At a routine check up in Dec 1974 the patient com plained of dizziness and an extremely low physical ca pacity A new medical examination revealed a systolic BP in the supine position varying from 75 to 115 mmHg from one moment to another The heart rate was 70 beats/ min In upright position the arterial pressure was now and then not measurable and the patient felt dizzy Examina tion of the heart revealed a broad left ventricular impulse A grade 3/6 (NYHA) systolic murmur was heard over the mitral area and was transmitted to the left axilla The in tensity of the murmur varied widely from one beat to an other

Peripheral pulse waves were recorded from the left thumb by a mercury in silastic strain gauge (2 5) The amplitude of the pulse waves varied considerably from one beat to another When the beats were induced by the artificial pacemaker the amplitude of the pulse waves was only about 30% of that caused by a sinus induced beat (Fig 1) None of the pacemaker induced beats were preceded by atrial activity A continuous recording for some minutes revealed a prononunced variation in both total volume and amplitude of the pulse wave of the thumb The beat to-beat pulse amplitude obviously varied in relation to the P–R intervals and also to variations in electrical induction of the ventricles (Fig 1) It was there fore decided to study the cardiac haemodynamics under various conditions

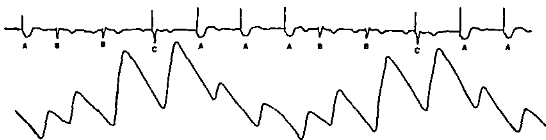


Fig 1 Synchronous variations in digital pulse volume caused by variations in induction of the ventricular con tractions After sinus induced beats the pulse amplitude is markedly increased compared with that recorded after pacemaker induced beats *A*=pacemaker induced *B*= sinus induced *C*=fused beats

Procedure

The patient was studied at rest and in the supine position A percutaneous right heart catheterization was performed with a double lumen Swan-Ganz catheter no 7 A catheter was also inserted percutaneously into the right femoral artery A mercury in silastic strain gauge was placed around the distal part of the left thumb for pulse detection

RESULTS

Idioventricular (IV) rhythm 53–58 beats/min
(Fig 2*A*)

A constant supraventricular rhythm with a P–R interval of 0 24 sec was recorded The patient had a hypokinetic circulation with an AV O_2-difference of 57 ml/l Normal pressures were recorded from the aorta lung artery and right atrium The mean pulse amplitude of the left thumb was 28 mm No ficant variation in amplitude was recorded dur ing the observation period of 17 min The intra arterial BP was also constant

Ventricular rhythm of 65 beats/min
(Fig 2*B*)

ECG showed both *IV* and pacemaker induced beats some of which were fused (Fig 2*B*) No pacemaker induced beats were preceded by atrial contractions Compared with the values recorded at an IV rhythm of 53–58 beats/min no significant changes in oxygen uptake minute volume or ar terial oxygen tension were recorded Stroke volume was 44 ml/min according to the Fick method which represents a decrease of 27% (Fig 3) The mean pulse amplitude was 18 mm 64% of that recorded at sinus rhythm There was a marked difference in amplitude between the pulses caused by a pace maker beat (mean 13 mm) and those caused by an IV beat (mean 29 mm) The amplitude of the former was sometimes only about 25% of the latter (Fig 2*B*)

Ventricular rhythm of 90 beats/min
(Fig 2*C*)

Most of the beats were pacemaker induced but some were fused or idioventricular Stroke volume was 34 ml corresponding to 57% of that recorded at a sinus rhythm of 55 beats/min When the right atrium contracted against closed valves there was a pronounced increase of the *a* waves (up to 18

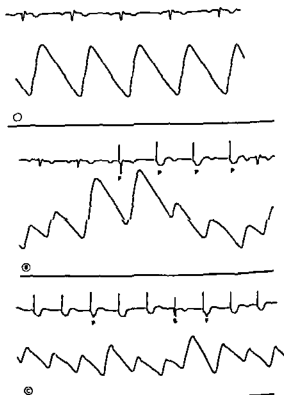


Fig 2 Variations in pulse amplitude of the left thumb The pulse amplitude is recorded at 53 (*A*) 65 (*B*) and 90 (*C*) beats/min Pronounced variations in digital pulse amplitude can be seen *F*=fused *P*=pacemaker induced *S*=sinus-induced beats

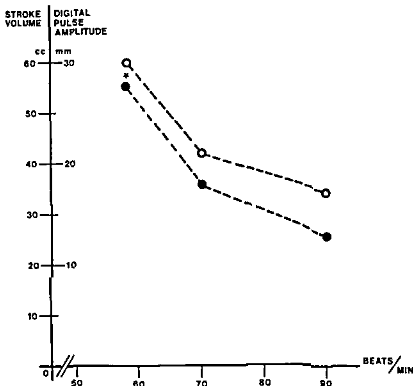


Fig 3 Variations in cardiac stroke volume and digital pulse amplitude Note the close correlation between these two measurements * sinus rhythm O=left ventricular stroke volume ●=pulse amplitude of the thumb

mmHg) Consequently the atrial contractions were very effective The mean digital pulse amplitude was 13 mm (range 7–20)

Change of stimulation rate

Implantation of a pacemaker with a stimulation rate of 45/min avoided competition but still protected the patient from syncopal attacks An essential improvement was experienced following this change in stimulation rate

DISCUSSION

Several authors have shown that atrial contractions are of great importance for cardiac output (8 9 10 13) The effect of atrial systole varies however from one patient to another Gillespie et al (4) found that it was of importance for the magnitude of stroke output in the normal heart but less valuable when the myocardium was diseased Other factors such as preclosure of the AV valves prior to ventricular contractions have also been shown to influence the haemodynamic variables (7 11 12)

Synchronous atrial activity prevents an initial regurgitation of blood during ventricular systole while asynchrony may result in partial incompetence in the AV valves (6 12) In our patient the marked decrease in digital pulse amplitude which was noticed when no P waves preceded the ventricular contraction for a reasonable interval was most probably due to a decrease in the amount of blood expelled into the central circulation This might have been due to a functional incompetence of the mitral valve during asynchrony between atrial and ventricular contractions causing a marked regurgitation of blood into the left atrium The wide variations in the intensity of the systolic murmur from her mitral insufficiency from one heart beat to the next speaks strongly in support of the above mentioned mechanism Such AV valvular incompetence has been reported in patients with asynchronous ventricular pacing (6)

In the present patient stroke volume decreased markedly when the artificial pacemaker was set at 70 and 90 beats/min A decrease in pulse amplitude was also noted which was almost parallel to that in stroke volume The recorded pulse amplitude which is related to the amount of blood expelled into the finger at every heart beat most probably reflects the beat to-beat stroke volume (1) How

ever further investigations are necessary to clarify the exact relationship between pulse amplitude in a toe or a finger and stroke volume It seems that measurement of toe or finger pulse amplitude with this simple non invasive strain gauge technique may be of clinical help in evaluating the effectiveness of the heart muscle and the haemodynamic consequences of measures such as cardiac pacing in patients with heart blocks

ACKNOWLEDGEMENTS

The study was supported by grants from the Swedish National Association against Heart and Chest Diseases and Martha and Gunnar Gordon s Foundation

REFERENCES

1 Bevegård S Karlof I & Resnekov L Effect of changes in ventricular rate on forearm blood flow and systemic arterial pressure in patients with artificial pacemakers Scand J Clin Lab Invest 32 245 1973

2 Fagrell B Vital capillary microscopy A clinical method for studying changes of the nutritional capillaries in legs with arteriosclerosis obliterans Scand J Clin Lab Invest (Suppl) 133 1973

3 Furman S & Escher D J W Principles and techniques of cardiac pacing p 165 Harper & Row New York 1970

4 Gillespie W J Greene D G Karatzas N B & deLee G J Effect of atrial systole on right ventricular stroke output in complete heart block Brit Med J 1 75 1967

5 Gundersen J Segmental measurements of systolic blood pressure in the extremities including the thumb and the great toe Acta Chir Scand (Suppl) 426 1972

6 Haas J M & Strait G B Pacemaker induced cardiovascular failure Hemodynamic and angiographic observations Am J Cardiol 33 295 1975

7 Jochim K The contribution of the auricles to ventricular filling in complete heart block Am J Psychol 122 639 1938

8 Karlöf I Hemodynamic effect of atrial triggered versus fixed rate pacing at rest and during exercise in complete heart block Acta Med Scand 197 195 1975

9 Samet P Bernstein W H Medow A & Nathan D A Effect of alterations in ventricular rate on cardiac output in complete heart block Am J Cardiol 14 477 1964

10 Samet P Castillo C & Bernstein W H Hemodynamic sequelae of atrial ventricular and sequential atrioventricular pacing in cardiac patients Am Heart J 72 725 1966

11 Samet P Jacobs W Bernstein W H & Shane R Hemodynamic sequelae of idioventricular pacemaking in complete heart block Am J Cardiol 11 594 1963

12 Sarnoff S J Gilmore J P & Mitchell J H Influence of atrial contraction and relaxation on closure of mitral valve Observations on effects of autonomic nerve activity Circ Res 11 26 1962

13 Winters W L Tyson R R Barrera F & Soloff L A Cardiac pacemaking II Physiological studies Ann Intern Med 62 220 1965

Acta Med Scand 202 335–336 1977

LETTERS TO THE EDITOR

Sir

I read with interest the paper by Bengtsson and Tibblin published in vol 196 p 93 1974 The results agree with ours However I think that the increase in uric acid in women aged 60 can be interpreted as a result of increased triglycerides observed in these ages because our previous work has shown that uric acid correlates well with triglycerides (1–4)

Manuel Judice Halpern

Department of Biochemistry Instituto de Ciencias Biomedicas de Lisboa Campo dos Martires da Pátria Lisboa Portugal

REFERENCES

1 Halpern M J Proc Int Col Lipoprot Lisbon 1975
2 Halpern M J & Pereira Miguel M J Lancet I 670 1973
3 — Ann Biol Clin 31 153 1973
4 — J Am Ger Soc 22 86 1974

Sir

On the relationships between age body weight serum triglycerides and serum uric acid

An association has been found between serum uric acid and body weight by us (2) as well as by other investigators An association has also been found between body weight and serum triglycerides (4) Body weight has been found to increase with age in women (3) A rise with age unrelated to a rise in body weight has also been found both for serum uric acid (2) and for serum triglycerides (4)

In a letter to the Editor Dr Halpern has proposed that the higher serum uric acid levels in upper ages might be due to higher serum triglycerides in these ages This proposal was based on the findings that a concomitant decrease or increase in serum uric acid and serum triglycerides could be achieved experimentally by reducing the glucine content in the food or loading with fructose (5) When relating serum uric acid to serum triglycerides in middle aged women a slight association has been found (1)

Our studies on serum uric acid levels (2) and serum cholesterol and serum triglyceride levels (4) were performed on the same population sample of women Dr Halpern s letter to the Editor has encouraged us to further studies of the relationships between body weight serum uric acid and serum triglycerides in these women Low degree linear correlations were found between the three variables studied (body weight–serum triglycerides r 0 18 body weight–serum uric acid r=0 26 serum triglycerides–serum uric acid r 0 23)

Table I presents serum uric acid levels for different ranges of serum triglyceride levels in the various age groups studied Serum uric acid seemed to rise independently of both age and increasing serum triglyceride levels

A multiple regression analysis was also carried out Age was included The following partial correlations were found between age and serum uric acid 0 19 between body weight and serum uric acid 0 25 between serum triglycerides and serum uric acid 0 23

We thus agree with Dr Halpern that serum triglyceride levels seem to have some influence on serum uric acid unrelated to age and body weight Whether such an influence is caused by the serum triglycerides themselves or by still other unrecognized factors related to the triglycerides remains to be settled

Calle Bengtsson *Elisabeth Tibblin*

Table I *Serum uric acid (μmol/l) in different ranges of serum triglyceride levels*

Age (y)	Serum triglyceride levels (μmol/l) 0 80–0 99			1 00–1 19			1 20–1 39			1 40–1 59		
	n	Mean	S D	*n*	Mean	S D	*n*	Mean	S D	*n*	Mean	S D
38	83	215	60	72	223	68	40	221	123	32	235	65
46	84	220	60	84	235	65	67	219	62	33	244	68
50	84	247	95	73	230	55	69	246	73	37	230	56
54	31	219	76	24	247	80	27	283	87	23	241	43
60	12	229	65	20	277	86	12	244	86	14	252	84
Total	294	227	74	273	235	67	215	238	86	139	238	62

REFERENCES

1 Benedek T G Correlations of serum uric acid and lipid concentrations in normal gouty and atherosclerotic men Ann Intern Med 66 851 1967
2 Bengtsson C & Tibblin E Serum uric acid levels in women An epidemiological survey with special reference to women with high serum uric acid values Acta Med Scand 196 93 1974
3 — Epidemiologiska synpunkter på overvikt hos kvinnor Läkartidningen 71 4189 1974
4 Bengtsson C Tibblin E Blohmé G & Gustafson A Serum cholesterol and serum triglycerides in middle aged women The study of women in Göteborg 1968–1969 Scand J Clin Lab Invest 34 61 1974
5 Halpern M J & Pereira Miguel M J Triglycerides and uric acid Ann Biol Clin 31 153 1973

Acta Med Scand 202 337–340 1977

Angina Pectoris and Myocardial Infarction

Claes Wilhelmsson J Anders Vedin Dag Elmfeldt Gosta Tibblin and Lars Wilhelmsen

From the Section of Preventive Cardiology Department of Medicine I Sahlgrenska Hospital Goteborg Sweden

ABSTRACT Angina pectoris was studied in a representative series of male patients ($n=504$) with a first myocardial infarction (MI) surviving the hospital stay The prevalence of questionnaire angina before MI was 28% and of effort induced chest pain alone 40% Of the patients with effort induced chest pain 72% retained symptoms also after MI No correlation with age was found Three months after and one year after infarction the prevalence of effort induced chest pain was 55% and 45%, respectively The patients with effort induced chest pain before MI had a somewhat more severe clinical course and a significantly higher death rate (15% versus 6%) than those without chest pain

Angina pectoris is an important symptom in ischemic heart disease Several publications have shown a high annual mortality among patients with angina pectoris (7) Among myocardial infarction patients stable angina pectoris is a common symptom before infarction (5)

The purpose of this report is to evaluate the importance of angina pectoris preceding myocardial infarction for long term prognosis

STUDY POPULATION AND METHODS

Since Jan 1st 1968 all cases of myocardial infarction (MI) occurring in Goteborg have been registered by a special Myocardial Infarction Register The register comprises 90% of all surviving diagnosed cases of MI in the city (1) After discharge from hospital all these surviving patients were systematically followed up at a special Post MI Clinic (2) The present study comprises men who suffered their first MI and were aged as follows During the years 1968 and 1969 all patients aged 55 years and below ($n=62$ and 66 respectively) 1970 and 1971 all patients aged 67 years and below ($n=171$ and 205 respectively) making a total of 504 male patients who survived their stay in hospital (Table I)

Before discharge from hospital the MI patients were contacted by one of the physicians of the Post MI Clinic (2) At the first appointment the patients were interviewed with respect to symptoms and case history In order to ensure uniform collection of data special forms were prepared The treatment of patients after discharge was standardized and uniform rules were established by means of regular meetings with the staff attending the patients Examinations took place at intervals required by the clinical situation and always 3 12 24 and 60 months after the MI (2)

Two groups of patients with chest pain were formed 1) Patients with chest pain defined according to Rose and Blackburn (10) 2) Patients with effort induced chest pain In this group all patients were included who reported chest pain upon physical exertion such as walking up a small hill or rapidly on level ground or upon less exertion Effort induced chest pain was used as the variable to describe the groups with and without chest pain Such factors as the effect of rest or intake of glyceryl trinitrate were not taken into consideration

For registration of dyspnea on exertion the questions recommended by Rose and Blackburn (10) were used The level of exertion was graded in the same way as regarding angina pectoris Dyspnea at onset of MI was based on the patient s statement at the first interview Left ventricular failure was considered to be present when more than occasional basal pulmonary rales and/or increased prominence of pulmonary vessels on X ray were noted in the patient s records Other conditions such as atrial fibrillation or atrioventricular block were considered to have occurred if they had been registered at any time during the stay in hospital

Approximately 50% of the patients were treated in a coronary care unit Blood samples for transaminase determinations (S-GOT and S-GPT) were taken Since the method of analysis was altered during the investigation and in order to obtain a uniform measure of maximum

---

Address for reprints C Wilhelmsson M D Department of Medicine I Sahlgrenska Hospital S-413 45 Goteborg Sweden

Table I *Patient series men with first MI 1968–71*

Age (y)	N
–39	14
40–44	32
45–49	93
50–54	144
55–59	97
60–64	81
65–67	43
Total	504

S-GOT and S-GPT for all patients absolute values for S-GOT are not stated in Table IV The relative frequency of patients with a value above that for the 4th quartile is given The occurrence of end points was established by the MI Register The same principles were used as for registration of the initial MI (1) The reinfarction was considered non fatal if the patient was discharged alive or survived 4 weeks All patients who died were examined post mortem and the cause of death was established according to WHO recommendations (1) Death certificates and necropsy reports were available for all deceased patients

For the statistical analysis χ^2 test was used for testing the difference between qualitative variables For continuous variables the mean standard deviation was calculated by the usual methods The statistical significance of differences between mean values was determined using Student s t test Differences were considered statistically significant for $p<0.05$

RESULTS

Chest pain before MI defined according to Rose and Blackburn (10) occurred in 28% of the patients and effort induced chest pain before MI in 40% No correlation with age was found for either group (Table II)

Before MI, 197 patients (40%) had effort induced chest pain Of these patients 72% retained the symptom (retained chest pain) and 20% became free from pain (chest pain abolished) 3 months after MI Among patients without chest pain before MI 36% developed chest pain (newly occurring chest pain) and 58% remained free from pain 3 months after MI Thus 3 months after MI 55% of the patients reported chest pain while 45% did not Twelve months after infarction 45% suffered from chest pain No correlation with age was found for newly occurring chest pain abolition of chest pain or retained chest pain at the 3 or 12 month check ups

Tables III and IV show certain preinfarction characteristics as well as variables registered in connection with the MI for patients with and without effort induced chest pain prior to the acute MI The patients with effort induced chest pain had dyspnea on exertion more often than those without chest pain and were also treated more often with digitalis (Table III)

Table IV shows the variables collected during the acute phase of the infarction which have proved to have the greatest ability to predict death during two years follow up after MI according to Vedin et al (14) Assessment of the data leads to the conclusion that the patients with effort induced chest pain had more severe acute MIs than the group without chest pain The former group had a significantly higher incidence of dyspnea at onset of MI and significantly larger relative heart volumes This group al

Table II *Angina pectoris according to Rose and Blackburn (10) and effort induced chest pain prior to MI (N=504)*

Age (y)	Angina pectoris according to Rose and Blackburn		Effort induced chest pain		Total
	n	%	n	%	
–39	4	29	4	29	14
40–44	4	13	7	23	31
45–49	23	25	40	44	90
50–54	42	30	62	44	141
55–59	24	25	38	40	96
60–64	31	38	31	38	81
65–67	13	30	15	35	43
Total	141	28	197	40	496

Table III *Preinfarction characteristics in men with first MI in the groups with and without effort induced chest pain prior to MI*

	Effort induced chest pain (N=197)		No chest pain (N=299)		
	n	%	n	%	p
Dyspnea on exertion	99	50	99	33	<0 05
Smokers	154	78	233	78	n s
History of hypertension	47	24	80	27	n s
Diabetes	12	6	11	4	n s
Treatment with digitalis	16	8	9	3	<0 05

Table IV *Variables recorded at onset and during hospital stay in men with first MI in the groups with and without effort induced chest pain prior to MI according to Vedin et al (14)*

	Effort induced chest pain (N=197)		No chest pain (N=299)		
	n	%	n	%	p
Dyspnea at onset	100	51	107	36	<0 05
Left ventricular failure	59	30	77	26	n s
AV block II+III	12	6	18	6	n s
Atrial fibril lation	16	8	21	7	n.s
S-GOT value (above highest quartile)	49	25	75	25	n s
	$\bar{X}$	s_x	$\bar{X}$	s_x	p
Relative heart volume (ml/m² BSA)	480	100	450	70	<0 05

so had a significantly higher incidence of vegetative symptoms at the onset of MI (82% versus 63%) and was significantly more often treated with diuretics while in hospital (35% versus 21%) than the group without chest pain No differences were found with respect to atrial or ventricular arrhythmias The duration of treatment in hospital was the same in the two groups

One year after MI a higher proportion of the patients with effort induced chest pain were receiving digitalis treatment (47%) and had higher cholesterol levels (270 mg/100 ml) than those without chest pain (31% and 250 mg/100 ml $p<0$ 05) These differences were not present 3 months after MI

Table V shows non fatal reinfarctions and deaths during two years follow up During the follow up period 15% of the patients with effort induced chest pain and 6% of the patients without chest pain had died There was no difference in the incidence of reinfarction between the two groups (16% versus 13%)

COMMENTS

In this investigation men who had survived a MI were subjected to special study Only male patients with primary MI were included to create a more homogeneous patient series

It is difficult to define angina pectoris unequivocally In epidemiological studies the interview technique proposed by Rose and Blackburn (10) is generally used This represents a reasonable compromise in the choice between high specificity and high sensitivity in a normal population (8) The present MI series has been studied using this method and compared with randomly selected population samples (3)

For follow up of MI patients another method was chosen the patients were asked whether they had chest pain on exertion There were several reasons for this A questionnaire with several questions is less suitable for repeated interviews since patients tend to alter their pattern of response (9) It is generally accepted that MI patients may have prolonged anginal attacks (>10 min) and may also have a higher tolerance for pain and therefore continue to exert themselves This means that the patient will not be recorded as having angina pectoris according to Rose and Blackburn s questionnaire In a postinfarction population angina pectoris accounts for a higher proportion of chest pain than in a normal population Therefore the simple definition of effort induced chest pain can be accepted despite its lower specificity if used in a normal population

The prevalence of angina pectoris prior to MI usually varies between 22% and 73% depending on definitions and the selection of study populations (4 6 12 13) Sievers (12) found that about 50% of 1411 patients with primary MI had had angina pectoris The prevalence of angina pectoris was not related to age and patients who had angina pectoris before the onset of MI tended to retain the symptom after MI These observations are in accordance

Table V *Reinfarctions and deaths during two years follow up in men with first MI in the groups with and without effort induced chest pain prior to MI*

	Effort induced chest pain (N=197)		No chest pain (N=299)		
	n	%	n	%	p
Reinfarctions	31	16	38	13	n s
Deaths	30	15	19	6	<0 05
Total	61	31	57	19	<0 05

with the findings of the present study In the Framingham Study 50% of postinfarction patients had angina pectoris and in 60% of them the symptom had appeared after the MI (5)

There are several possible explanations for the lower proportion of patients with effort induced chest pain after 12 months than after 3 months in the present study Patients with effort induced chest pain at the 3 month examination may have altered their way of life and adapted themselves to a lower level of activity Alternatively some patients with effort induced chest pain may have increased their physical fitness so that chest pain did not occur until higher levels of exertion Sanne et al (11) have shown that patients with angina pectoris who were tested on a bicycle ergometer 3 and 12 months after infarction developed chest pain at a higher working load after one year than at the 3 month check up even without regular supervised physical training Abolition of chest pain after acute myocardial infarction was reported in 15% of the patients in the Framingham Study (5) The reason for the absence of pain in these patients may be that there is no longer a zone of latent ischemia after the infarction

Our findings indicate that patients with effort induced chest pain prior to MI may have more advanced ischemic heart disease than patients without The patients with effort induced chest pain had a more severe and complicated clinical course hospital and developed a more extensive MI his substantially explains the worse prognosis post MI It is conceivable that a more advanced coronary disease as reflected by effort induced chest pain before infarction forms a substrate for a more extensive myocardial lesion

ACKNOWLEDGEMENTS

The study was supported by grants from the Swedish Medical Research Council and the Swedish National Association against Heart and Chest Diseases

REFERENCES

1 Elmfeldt D Wilhelmsen L Tibblin G Vedin J A Wilhelmsson C E & Bengtsson C Registration of myocardial infarction in the city of Goteborg Sweden A community study J Chron Dis 28 173 1975
2 — A Postmyocardial Infarction Clinic in Goteborg Sweden A follow up of MI patients in a specialized outpatient clinic Acta Med Scand 197 497 1975
3 Elmfeldt D Vedin A Wilhelmsson C Tibblin G & Wilhelmsen L Characteristics of representative male survivors of myocardial infarction compared with representative population samples Acta Med Scand 199 387 1976
4 Honey G E & Truelove S C Prognostic factors in myocardial infarction Lancet 1 1155 1957
5 Kannel W B & Feinleib M Natural history of angina pectoris in the Framingham Study Am J Cardiol 29 154 1972
6 Master A M & Jaffe H L Factors in onset of coronary occlusion and coronary insufficiency effort occupation trauma and emotion JAMA 148 794 1952
7 Reeves T J Oberman A Jones W B & Sheffield L T Natural history of angina pectoris Am J Cardiol 33 423 1974
8 Rose G Chest pain questionnaire In Comparability in international epidemiology (ed Acheson) Milbank Med Fund Q 43 part 2 32 1965
9 — Variability of angina some implications for epidemiology Br J Prev Soc Med 22 12 1968
10 Rose G A & Blackburn H Cardiovascular survey methods p 64 WHO Geneva 1968
11 *Sanne H Elmfeldt D Grimby G Rydin C &* Wilhelmsen L Exercise tolerance and physical training of non selected patients after myocardial infarction Acta Med Scand (Suppl) 551 1973
12 Sievers J Myocardial infarction Clinical features and outcome in three thousand thirty six cases Acta Med Scand (Suppl) 406 1963
13 Skall Jensen J 601 myocardieinfarcter p 1 Aarhuus Stiftsbogtrykkerie Århus 1967
14 Vedin A Wilhelmsen L Wedel H Pettersson B *Wilhelmsson C Elmfeldt* D & Tibblin G Prediction of cardiovascular deaths and non fatal reinfarctions after myocardial infarction Acta Med Scand 201 309 1977
15 Vedin A Wilhelmsson C Elmfeldt D Save-Soderberg J Tibblin G & Wilhelmsen L Deaths and non fatal reinfarctions during two years follow up after myocardial infarction Acta Med Scand 198 353 1975

Acta Med Scand 202 341–347 1977

Angina Pectoris in Young Patients Clinical Appraisal and Evaluation by Exercise, Atrial Pacing, and Myocardial Lactate Metabolism

M Rajasalmi and J Takkunen

From the Department of Internal Medicine Cardiovascular Division University of Oulu Oulu Finland

ABSTRACT Young patients with a symptom complex of angina pectoris constitute a problematic group in medicine Many of them require a detailed investigation By combining electrophysiologic and metabolic measurements with dynamic clinical experiments, one can distinguish with great probability between normal and pathological findings Exercise ECG, atrial pacing and simultaneous sampling of blood from artery and coronary sinus were undertaken in 57 patients aged 40 or less Although the patients were selected according to a history of effort angina results were normal in four and in seven others only one test was pathological The sensitivity was highest in atrial pacing (78%) and somewhat lower in exercise ECG (75%) and lactate extraction (67%) The specificity of the tests in series was about 53% Concordant results made a diagnosis of ischemic heart disease highly probable Discrepancies and probably false negative results in individual cases are evident The need for angiographic evaluation of coronary arteries and left ventricular function is clear The stepwise advance from the usual physical examination to more demanding investigations provides a possibility of classifying the patients according to the needs of an accurate medical appraisal

Chest discomfort and pain compatible with angina pectoris in connection with physical stress in young persons present a symptom complex that needs special attention in order to reach an adequate diagnosis This combination of a prognostically grave symptom and an active age is not only medically demanding but includes social and economic problems These are evident when the patient finds himself incapable of work Autopsy studies of acute and violent deaths (18 26) have shown atherosclerotic coronary changes even in young people On the other hand there are patients with angina pectoris and even with myocardial infarction in whom the coronary arteriography has been normal (5 6 8 17 22) Clinical studies in patients with anginal symptoms have led to the conclusion that atrial pacing does not contribute significantly to the diagnosis in patients submitted to the graded exercise test (24) From coronary arteriographies in asymptomatic patients with pathological exercise ECG it has been concluded that the usefulness of the exercise ECG is limited (3) The results of the clinical evaluation exercise tests and atrial pacing with the study of myocardial lactate metabolism have been concordant in reports by authors who find no room for coronary arteriography in the diagnosis of doubtful angina (19) In the studies cited the clinical history has been found highly significant for the diagnosis The patients were mostly over 40 years of age though some were younger However the differentiation of the etiology of angina pectoris is unclear in many young patients and even the results from the exercise studies are disputable

For this reason we have combined exercise ECG atrial pacing and a study of myocardial lactate extraction with the clinical findings in a group of patients aged 40 or less whose problem was an angina pectoris suspected of being caused by ischemic heart disease

STUDY POPULATION AND METHODS

Fifty seven patients 36 females and 21 males aged 40 or less with a history of angina pectoris (15) formed the

Table I *Age and sex distribution and weight/height ratios of the patients investigated*

Sex	N	Age (y)		Body weight/height	
		Mean	Range	Mean	Range
♀	36	34	21–40	0 372	0 293–0 465
♂	21	32	22–40	0 412	0 358–0 481

basis for the study The symptoms interfered with their subjective working capacity Angina pectoris was defined as a pain pressure or discomfort in the anterior area of the chest precipitated by effort and relieved within 5–10 min at rest Some of the patients also had prolonged symptoms but these were not related immediately to effort Except for the history the patients presented no objective evidence of ischemic heart disease at rest Hypertensive patients diabetics and patients with cardiomegaly or other overt cardiac disease were excluded after a thorough basic evaluation in the Outpatient Department Also patients with extracardiac causes of chest pain were excluded In the Cardiovascular Division the registration of symptoms was undertaken first so that they would not be biased by other investigations After a new physical examination the exercise test was conducted on the first day and the atrial pacing and lactate study on the following day

The age and sex distribution and relative weights of the patients are given in Table I The body weight/height ratios are within the same range as in Finnish population studies of the same age group

The multistage bicycle exercise ECG was begun with a load of 30 W increasing for women by 10 W/min and for men by 15 W/min Exercise was maximal until exhaustion or until anginal symptoms prevented further pedalling The bicycle was electrically braked (Godart Statham NV Bilthoven Holland) and the speed was 50 revolutions/min A conventional 12 lead ECG was registered before and at 3 and 5 min after exercise Patients were monitored during the exercise and a paper record was made every minute The electrodes were placed according to a modified CM_5 system (25) on the patient s forehead manubrium sterni high sacrum and on the thorax corresponding to the ECG lead V_5 Cuff BP was recorded every minute together with any changes in rhythm or conduction In the interpretation of the ST depression on the ECG a horizontal or downward sloping change of 0.5 mm or more during 60 min was regarded as pathologically significant (11)

The atrial pacing was performed with the aid of Zucker s catheter (29) placed in the coronary sinus The catheter tip was positioned as far as possible in the coronary sinus without preventing the blood sampling which would have meant wedging the vein This method was designed to yield as representative samples as possible for the measurement of lactate concentrations in the blood draining mostly the left ventricle This position normally had a suitably low threshold for the pacing with a Medtronic Model 5837 pulse generator The arterial samples were collected simultaneously with coronary sinus sampling The blood samples were immediately denaturated by a five fold amount of ice chilled trichloracetic acid (0 6-N) in closed tubes with a blender and transported to the laboratory for analysis by an enzymatic method (14) In the evaluation of lactate extraction the difference between the arterial and coronary sinus concentrations was calculated in per cent of the arterial concentration An extraction over 10% was regarded as normal A lower extraction or a net accumulation of lactate to the coronary sinus was considered pathological in keeping with previous studies (13)

The pacing was started up to 1 min after an i v injection of 0 6 mg atropine which was given to prevent interference from Wenckebach s phenomenon The heart rate was brought up to 170/min in 30 sec Blood samples were taken and a 12 lead ECG was recorded before at 2 and 4 min during and at 3 and 5 min after the pacing which lasted for 4 min A 3 lead ECG from leads V_4–V_6 was recorded every minute during pacing and just at the transition from pacing to sinus rhythm to permit a comparison of the ST segment depression during and immediately after pacing when any distortion from the pacing artifact had been eliminated (23)

RESULTS

Angina pectoris

Seven men and 19 women had a history of early coronary heart disease in their family i e in immediate members of the family aged less than 50 among men and less than 60 among women (Table II) Fourteen women and 6 men also complained of prolonged chest discomfort The interval from the onset of symptoms to the examination varied from 0 5 to 8 years with three exceptionally long histories The mean duration of the symptoms was the same for men and women More anginal symptoms were noted in the patients with pathological findings (Table III) All the patients felt the pacing and 22 women and 12 men developed angina pectoris The pain mostly began in the third minute

Table II *Clinical findings from patient history and the duration (mean±S D) and type of angina*

	Family history of early CHD	Effort angina	Prolonged angina after stress	Duration of symptoms* (y)
Females	19	36	14	2 3±1 7
Males	7	21	6	2 3±1 6

* Two women and one man have been excluded from the calculation owing to an exceptionally long history of angina pectoris (10 15 and 18 years)

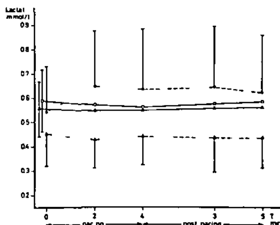


Fig 1 Arterial and coronary sinus lactate concentrations before during and after atrial pacing O=arterial values in patients with pathological lactate extraction ●=the corresponding coronary sinus concentrations Δ=arterial concentration in patients with normal lactate extraction ▲=the corresponding coronary sinus concentrations All 57 patients are included and S D are indicated The difference between coronary sinus concentrations was statistically significant ($p<0\ 01$)

and was relieved spontaneously when pacing ended

Lactate study

Various results from the lactate study are presented in Table IV In 21 patients there was both a low extraction and a net accumulation of lactate in the coronary sinus blood All patients with pathological findings had abnormal results in 2–4 samples Normal lactate extraction was found in 12 women and 7 men The normal and pathological lactate concentrations at rest and during and after pacing are depicted in Fig 1 The arterial concentrations did not differ greatly in normal and pathological findings If one arterial value deviated more than 10% from others in the same patient it was discarded In coronary sinus blood the variation was naturally greater and largest in pathological findings The range of lactate extraction was accordingly wider here The distribution of the highest normal and pathological lactate extraction values is presented in Fig 2 correlated to the ST segment depression in the same patient The ST changes have been interpreted with an accuracy of 0 5 mm There was no clear trend to any correlation between the changes in the lactate study and ECG Neither does the sex distribution in the figure indicate any difference between men and women

Table III *Results from ECG and lactate studies*

A=changes from normal not compatible with coronary heart disease P=pathological finding in test N=no pathological finding in test or normal

	Resting ECG		Exercise ECG		Pacing ECG		Lactate study	
	A	N	P	N	P	N	P	N
Females	21	15	31	5	32	4	24	12
Males	10	11	13	8	13	8	14	7
With anginal pain								
Females	–	–	15	2	20	2	16	7
Males	–	–	5	4	7	5	7	3

ECG findings

Half of the patients had abnormal configurations in the resting ECG (Table III) These changes were mostly variable so that the same patient had registrations from about normal to obviously divergent The transient changes were mostly in the ST T segment in the chest leads Four patients had left axis deviation Two had a transitory RBBB One patient demonstrated repeatedly negative T waves during orthostatic stress No patient had changes compatible with previous myocardial infarction All had sinus rhythm and normal atrioventricular conduction at rest Five female and eight male patients had normal results in the exercise ECG and four and eight respectively in the pacing ECG The amount of pathological findings in the lactate study (Table III) is of the same magnitude in both sexes if allowance is made for the total numbers of men and women

The total numbers of normal and pathological findings in the various tests are presented in Table V The sensitivity of exercise ECG pacing ECG and lactate study is 75 5 78 9 and 66 7% respectively

Table IV *Lactate extraction and accumulation in relation to the type of possible abnormality and the sex of the patient*

	Females	Males
Lactate extraction below 10% and accumulation of lactate during pacing	13	8
Only extraction below 10%	7	1
Lactate accumulation during pacing	4	5
Normal results in lactate study	12	7

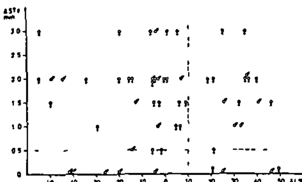


Fig 2 Correlation between ST segment depression (*ΔST*) and myocardial lactate extraction (*AL*) = The normal limits used in this study

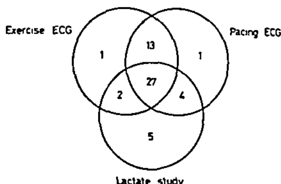


Fig 3 Distribution of the 57 patients according to findings in exercise ECG pacing ECG and lactate study Four patients had normal results and are not included here

An individual patient could have one, two or *three pathological findings The numbers of the* various combinations of pathological findings in the exercise ECG pacing ECG and lactate study are presented in Fig 3 In 27 patients about half of the total all three results were pathological Only pacing and exercise ECGs were pathological in 13 patients The result of the lactate study was congruent with only exercise ECG in two patients and with only pacing ECG in four Four patients with subjective symptoms compatible with angina pectoris had normal results in these investigations according to criteria used

DISCUSSION

The men in this study group had a slightly lower mean age than the women (32 vs 34) but the sex distribution is the reverse of that seen in the age group 41–60 with angina pectoris or myocardial infarction A clear family history was found in half of the women and in one third of the men Only first-degree family members under 50 (men) or 60 (women) were considered in this respect because coronary heart disease is very common at higher ages in Finland The mean duration of symptoms was quite short Three patients had an exceptionally long history A man of 28 had had his first attack in the form of chest pain and fainting at the age of 10 and later he had also had the same symptoms during hard work During exercise ECG he developed a second degree AV block The other two with a long history were women One of them (age 38) had had her first effort pains in the chest about 15 years ago but she had an even longer history of vascular headache The other woman (age 30) with coronary deaths in the immediate *family had suffered from angina pectoris* for 10 years

The definition of angina pectoris used here is in accordance with Hurst and Logue (15) Apart from angina of effort 20 of the 57 patients had a subjective symptom complex of prolonged chest pain and/or discomfort beginning shortly or some hours after the stress and lasting for a long time even hours at rest and in many cases interfering with sleep at night The prolonged symptoms were equally common in both sexes This type of prolonged pain is described in connection with cardiomyopathy (28) and may represent myocardial failure of ischemia Conclusions about its etiology can not be drawn from ECG or lactate studies It was present in 12 patients with pathological lactate extraction and in 8 with a normal result

There is no doubt today about patients who have a myocardial infarction with open coronaries In young women clear symptoms of myocardial ischemia have been found without coronary changes at arteriography (5 17 22) The patholog-

Table V *Sensitivity of various tests performed*

Owing to lack of absolute criteria for the existence of ischemic heart disease the specificity of the tests has not been calculated

Results	Exercise ECG	Pacing ECG	Lactate study
Pathological	44	45	38
Normal	13	12	19
Sensitivity* (%)	75.5	78 9	66 7

$^{*}\ \dfrac{\text{Pathological results}}{\text{Normal + pathological results}} \times 100$

cal and normal findings in the present patients are given together with their subjective feeling of angina pectoris during exercise and pacing in Table III There are 36 women and 21 men and normal findings in the exercise and pacing ECGs are relatively more common among the men In the lactate study one third of both sexes had normal results The feeling of angina pectoris during exercise and pacing was very variable In relation to their total number women were about twice as sensitive as men In general the sensation which the patient felt was the same as the one he was being investigated for In some patients the mere pacing was experienced as quite unpleasant and distressing Angina was registered when there was pain Five men and 11 women developed symptoms during both exercise and pacing Of these four men and eight women had pathological findings in the lactate study In the verified coronary patients atrial pacing has induced angina pectoris in about 1/3–2/3 (12 21 23)

We could not arrive at an anatomical diagnosis of the coronary arteries in the present patients but their sex distribution is the opposite of that for the natural history of coronary heart disease in patients over 40 After the age of 40 men are more prone to get a severe disease The first patients in this study were investigated more than two years ago and they all are still alive No clear accumulation of risk factors was found apart from the family history To clear up the anatomical and possible functional disturbances an angiographic evaluation of the coronary arteries and left ventricle is indicated in many of these patients

In many previous investigations of the myocardial lactate metabolism the same criteria as we used have been considered to indicate myocardial ischemia (12 13 23) The zonal mode of ischemia and the arrangement of the venous drainage of the heart are two factors that interfer with the reliability of the results owing to the mixing of venous blood in the coronary sinus The arterial concentrations of lactate and free fatty acids have been shown to affect the results (16) In another study (7) no significant difference was found in free fatty acids triglycerides glucose pyruvates potassium or total CO_2 between normal people and angina pectoris patients during pacing In the latter two studies the heart rate was increased more slowly than in our procedure Our patients were studied in the fasting state in the morning without physical exercise and with minimal other derangement so the situation favored a stable free fatty acid level though this was not determined The possible shunting caused by the pacing itself has also been noted by critics of the method In 20 patients we measured the electrolyte and oxygen concentrations from arterial and coronary sinus blood before during and after pacing The values were normal and did not change in agreement with previous results (7 12 21) This practically excludes a degree of shunting through the coronary vasculature that would affect the results of our experiments But this criticism may touch on the reason why it is not possible to verify the myocardial ischemia more accurately by means of the lactate study

A comparison of the results in Table IV and in Figs 1 and 2 shows that there is a clear distinction between the normal and the pathological values with multiple sampling before during and after pacing Clearly pathological values with low extraction and a net accumulation of lactate were found in one third of the patients and normal values in one third Less than one third had only low extraction or only a production of lactate in coronary sinus blood and then the changes were pathological in 2–4 consecutive samples The method can be held reliable and useful if its pitfalls are taken in consideration

The reliability of ECG studies for coronary heart disease and myocardial ischemia has been widely discussed (3 11 19 24 25) In our study an ST depression of 0 5 mm or more was taken to indicate a pathological finding if the coronary arteriogram is used as the criterion of existing disease (11) the results show that this examination is highly sensitive However positive correlations between arteriograms and autopsy findings vary from 67 to 100% (9 10) There is clearly a possibility of over diagnosis when an ST depression of 0 5 mm is used as a criterion of ischemia Fig 2 shows that four patients were on this limit and if it is changed to 1 mm there would have been six two of whom had normal lactate extraction Most patients exercised until a heart rate 10–20 below their physiological maximum according to age what is held to be a criterion of adequate stress (27) The pacing rate of 170/min also meets this requirement The patients complaints agree with the results in all but four (2 women and 2 men) whose results were normal and who have since been free from

symptoms All the patients in the study had a diagnosis of angina pectoris on a clinical basis so there is no symptom free group And as there are no absolute indicators of coronary heart disease it is not possible to calculate the specificity of the tests accurately (20) though their sensitivity is presented in Table V The results are comparable with previous investigations (4 12 19 23 24) One can also use the concept of the tests in series, where a person is considered positive only if all the tests in a series are positive (20) In our study, all tests are positive in 27 patients (Fig 3) leaving 30 who are negative in this sense i e an approximately 53% ability to identify those who do not have the disease For exercise ECG this result is in agreement with a previous investigation (1) On the other hand, it has been found that even among patients with major obstructive lesions in one or more coronary vessels only 17/21 developed angina pectoris during pacing and three of these had normal lactate values (23) In terms of tests in series one must therefore suspect that some of our patients with minor findings are falsely negative This does not mean that the lactate study is of no value For example patients with a very low exercise capacity, short exercise time and rapidly achieved maximal pulse rate will not necessarily have any ST changes or angina pectoris but these findings are clear in atrial pacing and lactate values are pathological

While we agree that a simple exercise test may be sufficient in most instances (24) several situations call for confirmation On the basis of our results we consider that ST changes and pathological lactate findings in coronary sinus blood are an expression of myocardial ischemia even though the results were congruent in only about half of our patients The selection criteria used may be fallacious but because the diagnosis of angina pectoris is primarily an anamnestic judgement we must also accept the possibility of making wrong conclusions An adequate solution requires further knowledge about the patient and a longer follow up in order to arrive at the medically appropriate decisions

REFERENCES

1 Åstrand I & Lundman T The exercise electrocardiogram in coronary heart disease Its prognostic value Scand J Clin Lab Invest 22 301 1968

2 Balcon R Hoy J Malloy W & Sowton E Haemodynamic comparisons of atrial pacing and exercise in patients with angina pectoris Br Heart J 31 168 1969

3 Borer J S Brensike J F Redwood D R Itscoitz S B Passamani E R Stone N J Richardson J M, Levy R I & Epstein S E Limitations of the electrocardiographic response to exercise in predicting coronary artery disease N Engl J Med 293 367 1975

4 Boudoulas H Cobb T C Leighton R F & Wilt S M Myocardial lactate production in patients with angina like chest pain and angiographically normal coronary arteries and left ventricle Am J Cardiol 34 501 1974

5 Bourassa M G & Pasternac A Douleurs angineuses avec coronaires normales ou peu lésées Schweiz Med Wochenschr 104 1605 1974

6 Cheitlin M D McAllister H A & de Castro C M Myocardial infarction without atherosclerosis JAMA 231 951, 1975

7 Daubert J C Feuillu A Pony J C & Gouffault J Etude comparative des critères metaboliques de l ischémie aigue en clinique humaine Arch Mal Coeur 68 599 1975

8 Dwyer, E M Wiener L & Cox J W Angina pectoris in patients with normal and abnormal coronary arteriograms Am J Cardiol 23 639 1969

9 Forsberg S Å Alestig K Bjure J Haggendahl E Paulin S Varnauskas E & Werkö L Post mortem coronary arteriographic clinical and electrocardiographic findings in 80 patients investigated with coronary arteriography Acta Med Scand 189 463 1971

10 Fournier C Barrillon A Paraiso N Saudemont J P & Gerbaux A Comparaison entre les aspects angiographiques et les lésions anatomiques des artères coronaires Arch Mal Coeur 68 691 1975

11 Friesinger, G C & Smith R F Correlation of electrocardiographic studies and arteriographic findings with angina pectoris Circulation 46 1173 1972

12 Helfant R H Forrester J S Hampton J R Haft J I Kemp H G & Gorlin R Coronary heart disease Differential hemodynamic metabolic and electrocardiographic effects in subjects with and without angina pectoris during atrial pacing Circulation 42 601 1970

13 Herman M V Elliott, W C & Gorlin R An electrocardiographic anatomic and metabolic study of zonal myocardial ischemia in coronary heart disease Circulation 35 834 1967

14 Hohorst H J L-(+)-Lactat Bestimmung mit Lactat Dehydrogenase und NAD In Methoden der enzymatischen Analyse (ed H U Bergmayer) pp 1425–1429 Verlag Chemie Weinheim 1970

15 Hurst J W & Logue R B The clinical recognition and medical management of coronary atherosclerotic heart disease In The heart (ed J W Hurst R B Logue R C Schlant & N K Wenger) pp 1038–1132 McGraw Hill New York 1974

16 Kaijser L Carlson L A Eklund B Nye E R Rössner S & Wahlqvist M L Substrate uptake by the ischaemic human heart during angina induced by atrial pacing In Effect of acute ischaemia on myo-

cardial function (ed M F Oliver D G Julian and K W Donald) pp 223–233 Churchill Livingstone Edinburgh and London 1972
17 Kemp H G Vokonas P S Cohn P F & Gorlin R The anginal syndrome associated with normal coronary arteriograms Report of six years experience Am J Med 54 735 1973
18 Kuller L Cooper M & Perper J Epidemiology of sudden death Arch Intern Med 129 714 1972
19 Livesley B & Oram S Diagnosis of doubtful angina Comparison of radiotelemetric exercise electrocardiogram with lactate content of coronary sinus blood after pacing Lancet 1 1461 1973
20 Mausner J S & Bahn A K Criteria for evaluation of screening tests In Epidemiology an introductory text pp 242–248 Saunders Philadelphia 1974
21 Neill W A Myocardial hypoxia and anaerobic metabolism in coronary heart disease Am J Cardiol 22 507 1968
22 Oliver M F Ischaemic heart disease in young women Br Med J 34 253 1974
23 Parker J O Chiong M A West R O & Case R B Sequential alterations in myocardial lactate metabolism S T segments and left ventricular function during angina induced by atrial pacing Circulation 40 113 1969
24 Piessens J van Mieghem W Kesteloot H & de Geest H Diagnostic value of clinical history exercise testing and atrial pacing in patients with chest pain Am J Cardiol 33 351 1974
25 Redwood D R & Epstein S E Uses and limitations of stress testing in the evaluation of ischemic heart disease Circulation 45 1115 1972
26 Rissanen V Aortic and coronary atherosclerosis in a Finnish autopsy series of violent deaths Ann Acad Sci Fenn (Med) 155 39 1972
27 Roskamm H & Samek L Die Beziehungen zwischen Koronarangiogramm und Belastungs EKG Dtsch Med Wochenschr 100 2538 1975
28 Takkunen J & Huhti E Nonobstructive cardiomyopathy in Northern Finland Ann Clin Res 2 102 1970
29 Zucker I R Rothfeld E L & Bernstein A A new multipurpose cardiac catheter Am J Cardiol 15 45 1965

Acta Med Scand 202 349–356 1977

Myocardial Scintigraphy as a Supplementary Diagnostic Tool in Heart Disease

Arne Gustafson Ingemar Larsson Lars Olsson
Sven Eric Svensson and Håkan Westling

From the Departments of Cardiology Radiation Physics and Clinical Physiology University Hospital Lund Sweden

ABSTRACT Myocardial scintigraphy with cesium 131 and thallium 201 was performed in 191 patients Previous myocardial infarctions localized to the anterior and lateral wall of the left ventricle were correctly diagnosed with both radionuclides Inferior and posterior infarctions were only detected when thallium was used In patients with non informative ECG changes like bundle branch block non specific ST T changes or with atypical symptoms myocardial imaging made an essential contribution to the establishment of a correct diagnosis The potential value of myocardial imaging in patients with valvular heart disease and in cardiomyopathy is described

Radionuclides are valuable tools for obtaining morphological and functional images of various organs in the body The value of these methods is beyond dispute in the diagnosis of thyroid pulmonary and renal diseases As to the heart radionuclides have mostly been used for the determination of coronary blood flow (6 12) and for the detection and quantification of intracardiac shunts (7) More than ten years ago it was shown that the potassium analogue cesium 131 accumulated in the myocardium and that infarcted areas appeared as cold spots (3) However not until the last few years have radionuclides been used to any major extent for labelling infarcts (8 10) and healthy myocardium (14 15)

The purpose of the present paper is to report our experience of myocardial scintigraphy with cesium 131 and thallium 201 in the evaluation of patients with known or suspected heart disease Emphasis will be laid upon the potential value of scintigraphy as a complementary diagnostic tool in patients in whom other non invasive methods have failed to give an accurate diagnosis

PATIENTS

Only patients with known or suspected heart disease were investigated Indications for scintigraphy in the latter group were non informative ECG changes (bundle branch block pacemaker ECG preexcitation ST T changes and arrhythmias of unknown cause) enlarged hearts and symptoms of obscure origin like breathlessness and atypical chest pain

The study population consisted of 191 patients investigated from Feb 1974 until June 1976 They were divided into six groups on the basis of anamnestic ECG X ray and auscultatory findings The groups are presented in Table I

Group I Myocardial infarction This group was composed of 78 patients (12 females) with a mean age of 53 years (range 13–74) They had previously suffered from proven infarction either transmural ($n=51$) indicated by pathological Q waves or subendocardial ($n=27$) diagnosed by history in combination with ST T changes and/or enzyme changes

Cesium 131 was used in 26 patients with transmural and in 20 with subendocardial infarction while the corresponding numbers investigated with thallium-201 were 25 and 7

Group II Non informative ECG changes This group consisted of 53 patients (11 females) with a mean age of 51 years (range 17–75) They were usually referred to myocardial scintigraphy to decide whether they had sustained infarction or not

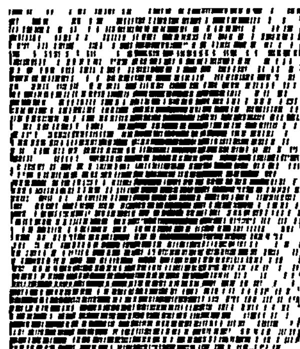
Twenty five patients were investigated with cesium 131 and 28 with thallium 201

Group III Cardiomyopathies This group consisted of 15 patients (4 females) mean age 45 years (range 19–65) Nine were classified as having hypertrophic and 6 congestive cardiomyopathy Seven of the patients had asymmetric septal hypertrophy

Five patients were investigated with cesium 131 and 10 with thallium 201

Group IV Cardiomegaly Two male patients 49 and 33 years of age made up this group Their ECGs were normal and heart volumes 600 and 660 ml/m² BSA respectively

Both patients were investigated with thallium 201



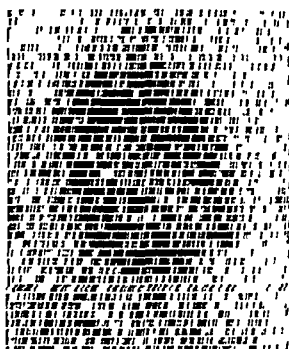


Fig 1 Cesium-131 scintigrams in frontal projection of a probably normal heart (to the left) and in a patient with anteroseptal infarction (to the right) The infarction appears as a zone with decreased uptake

Group V Angina pectoris This group comprised 18 patients (17 males) with a mean age of 50 years (range 38–62) the female patient was 56 years old All had chest pain typical or atypical for angina pectoris None of the had suffered documented myocardial infarction none had ECG changes conclusively showing ial infarction

Seven patients were investigated with cesium 131 and 11 with thallium 201

Group VI Valvular heart disease This group consisted of 25 patients (4 females) with a mean age of 52 years (range 34–62) Indications for scintigraphy were chest pain Q waves on the ECG without clinical correlation bundle branch block or advanced ST T changes Twenty patients had aortic and five mitral valvular disease

Eight patients were investigated with cesium 131 and 17 with thallium 201

METHODS

Radionuclides

Cesium-131 was obtained from Radiochemical Centre Amersham England It has a physical half life of 9 7 days and decays exclusively by orbital electron capture directly to xenon 131 The energy of the resulting radiation is about 30 keV

Thallium-201 was obtained from Philips-Duphar Petten Holland It has a physical half life of 73 5 hours and decays by electron capture with emission of γ-rays of 135 and 167 keV in about 10% of its disintegrations but measurements were made on the characteristic mercury X rays of 69–80 keV which are about 95% abundant

Cesium 131 1 0 mCi was given i v to the fasting patient Scintigraphy was performed 2–3 hours later Thallium 201 1 5 mCi was given i v with the fasting patient standing during the injection Scintigraphy was performed within 1 5 hours after the injection

Table I *Division of the patients into six groups*

Figures within parentheses=no of patients investigated with thallium 201

Group		No of pats
I	Myocardial infarction	
	Transmural	51 (25)
	Subendocardial	27 (7)
II	Non informative ECG changes	53 (28)
III	Cardiomyopathies	
	Hypertrophic	9 (8)
	Congestive	6 (?)
IV	Cardiomegaly	2 (2)
V	Angina pectoris	18 (11)
VI	Valvular heart disease	
	Aortic	20 (13)
	Mitral	5 (4)
	Total	191

Instrumentation

A conventional rectilinear scanner (Picker Magna Scanner V) was used for scintigraphy with cesium 131 The scanner was provided with a focusing collimator (3 265) and crystal (Ø 5×4") The patients were investigated in anterior posterior and left anterior oblique projections A gamma camera (Searle Pho/Gamma HP) interfaced to a digital system (Intertechnique Cinescintigraphic system) was used for thallium 201 Myocardial images were recorded with a converging low energy collimator for 5 min with a 35% window set symmetrically around the X ray peaks Images were obtained in 6 projections with the patient supine (right anterior oblique 30 anterior posterior left anterior oblique 22 45 and 67 and left lateral 90°)

RESULTS

Myocardial infarction

Twenty six patients with previous transmural myocardial infarctions were investigated with cesium 131 (Table II) One patient had suffered two infarctions The localization of the infarctions was based on the ECG The scintigrams were judged as normal questionable or pathological Eighteen anterior or anterolateral infarctions were all detected by scintigraphy Only 2 of 8 inferior wall infarctions were diagnosed with cesium scintigraphy A posterior wall infarction in one patient was not detected A normal scintigram with cesium 131 and a scintigram with an anteroseptal infarction are shown in Fig 1

Myocardial images were obtained with thallium 201 in 25 patients with previous transmural myocardial infarction (Table II) Two patients had suffered two infarctions Localization of infarctions was based on the ECG and the scintigraphy was evaluated as for cesium Scintigraphy with thallium diagnosed correctly all 10 infarctions localized to the anterior and lateral walls of the left ventricle Of 17 infarctions localized to the inferior wall and adjacent areas 13 were correctly diagnosed 2 were not detected and in 3 cases the scintigram was questionable A normal scintigram with thallium 201 and a scintigram with a posterior wall infarction are shown in Fig 2

Twenty patients with previous subendocardial anterior infarctions were studied with cesium 131 In 13 of them an obvious defect appeared in the scintigram In the other 7 the scintigram was judged as normal or questionable

Only 7 patients with previous subendocardial infarctions were investigated with thallium 201 In 2 patients obvious defects were visualized although their ECG was normal The scintigrams were regarded as normal in 2 patients and questionable in 3

Table II *Documented transmural infarctions diagnosed by myocardial scintigraphy with cesium 131 and with thallium 201*

Infarct localization	No of infarcts	Not detected	Questionable	Diagnosed
Cesium 131				
Extensive anterior wall infarction	3			3
Anteroseptal	7			7
Anteroapical	4			4
Anterolateral	4			4
Inferior wall infarction	8	6		2
Posterior	1	1		
Thallium 201				
Extensive anterior wall infarction	1			1
Anteroseptal	4			4
Anteroapical	2			2
Anterolateral	2			2
Inferior wall infarction	10	1	2	7
Inferolateral	4	1		3
Inferoposterior	3		1	2
High lateral	1			1

Non informative ECG changes

The 53 patients in this group were referred to scintigraphy usually to decide whether or not they had sustained an infarction

Of 25 patients investigated with *cesium 131* 12 had an area of reduced uptake in the heart indicating myocardial destruction probably due to infarction Eight of these patients had LBBB 2 had an artificial pacemaker one had non informative ST T changes and one QS complexes in precordial leads but no history of myocardial infarction In the 13 patients without scintigraphic signs of infarction 2 had LBBB 2 Q waves in precordial leads suggesting anterior infarction but without history and the remainder had non informative ST T changes The value of scintigraphy to elucidate the cause of pathological Q waves in precordial leads is illustrated in Fig 3 Neither of these two patients was investigated with invasive methods

Twenty-eight patients were investigated with *thallium 201* 8 had a localized area of reduced uptake ECG showed ventricular conduction defects

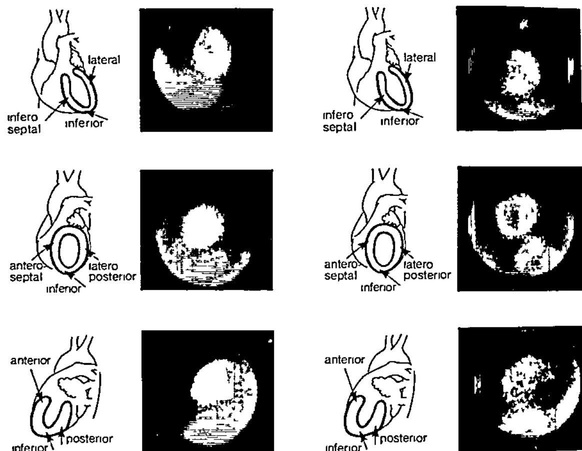


Fig 2 Thallium 201 scintigrams of a probably normal heart (to the left) and of a patient with an infarction localized mainly to the posterior wall (to the right) The projections are from the top frontal left anterior oblique (45°) left lateral (90°)

in 7 of these 8 patients The scintigrams were classified as probably normal in 10 of the remaining 20 patients and signs of ventricular hypertrophy or dilatation were seen in 10 (Fig 4)

Cardiomyopathies

Six patients with congestive cardiomyopathy were investigated with scintigraphy Mean roentgenological heart volume was 787 ml/m² BSA (range 600–980) Left ventricular angiography and coronary angiography performed in 3 patients showed diffuse hypokinesia and normal coronary arteries Autopsy showed normal coronary arteries in a fourth patient

Reduced uptake indicating myocardial destruction was seen in 5 of the 6 patients The areas of reduced uptake were similar in appearance to those in myocardial infarction

Nine patients with hypertrophic cardiomyopathy were investigated Seven of them had asymmetric septal hypertrophy The diagnosis was verified by angiography and/or non invasive methods (pulse recording and echocardiography)

The thallium scintigram was judged as normal in one patient with asymmetric septal hypertrophy and showed increased uptake in the septal and apical areas in 8 patients However the findings were never so typical that a correct diagnosis could be made solely from the scintigraphic investigations

Cardiomegaly

In the 2 patients in this group the cardiomegaly had been discovered by routine chest X ray Physical findings and ECG were normal

The thallium scintigrams were normal except for an increased size of the left ventricle

Angina pectoris

This group was divided into two subgroups one comprising patients with normal ECG at rest and

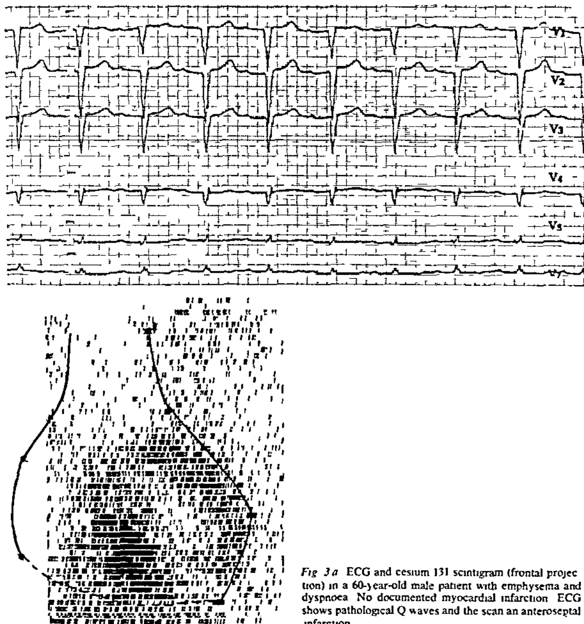


Fig 3a ECG and cesium 131 scintigram (frontal projection) in a 60-year-old male patient with emphysema and dyspnoea No documented myocardial infarction ECG shows pathological Q waves and the scan an anteroseptal infarction

the other patients with pathological ECGs but without changes typical for infarction

Normal ECG Three patients with normal ECGs were investigated with cesium 131 the scintigrams were normal in 2 They were further investigated with angiography and coronary angiography which gave almost normal findings In the third patient the scintigrams was of borderline significance However no further invasive investigation was performed

Seven patients with normal ECGs were investigated with thallium 201 Four of them had probably normal scintigrams and one had a normal coronary and left ventricular angiography The remaining 3 patients showed reduced uptake on the scintigram they had pathological exercise tests and coronary angiography showed occlusion of one vessel

Pathological ECG Eight patients with pathological ECGs were investigated with cesium 131 or thallium 201 Five of them had scintigraphic defects probably due to infarction and the results were inconclusive in three

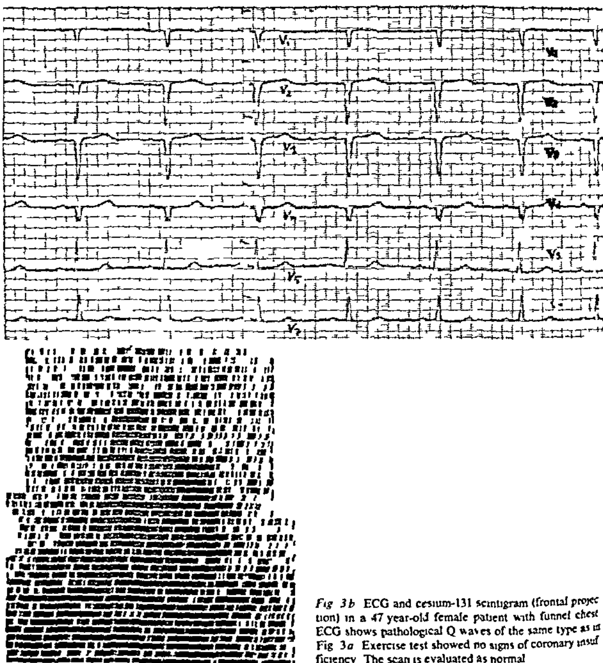


Fig 3b ECG and cesium-131 scintigram (frontal projection) in a 47 year-old female patient with funnel chest ECG shows pathological Q waves of the same type as in Fig 3*a* Exercise test showed no signs of coronary insufficiency The scan is evaluated as normal

Valvular heart disease

None of the 25 patients in this group had suffered a myocardial infarction according to usual criteria.

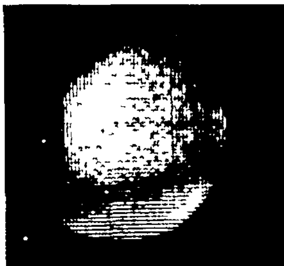
Of 20 patients with aortic valvular disease 13 had abnormal ECG changes which could indicate myocardial infarction or were non informative Reduced uptake was noted in 4 of these patients while 9 had no defects In 7 patients the indication for scintigraphy was usually chest pain Two of these patients had scintigraphic defects indicative of myocardial destruction

Of 5 patients with mitral valvular disease 3 had scintigraphic defects indicating a myocardial destruction which was not diagnosed with ECG

DISCUSSION

A series of radionuclides have been used for labelling the healthy myocardium e g ^{131}Cs ^{129}Cs ^{134}Cs^{m} ^{43}K ^{13}N ammonia ^{81}Rb ^{86}Rb ^{81}Kr^{m} and ^{201}Tl (5)

Most of these agents are impracticable or impos-



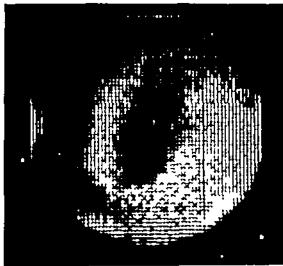


Fig 4 Scintigrams with thallium 201 in left lateral projections (90°) in two patients with non informative ECG changes indicating left ventricular hypertrophy Homogenous left ventricular hypertrophy (to the left) and left ventricular dilatation (to the right) Relative heart volumes were 1 170 and 800 ml/m² BSA respectively

sible to use in clinical routine work due to unsuitable energy spectra or short physical half life In our hospital only two isotopes were available for myocardial imaging ^{131}Cs and ^{201}Tl ^{131}Cs originally introduced for myocardial imaging by Carr et al (3) is suitable for examination of the anterior and lateral walls of the left ventricle In the present study all transmural anterior wall infarctions were detected with cesium 131 and the rectilinear scanner Furthermore subendocardial infarction localized to the anterior wall in 20 patients was diagnosed in 13 Our findings on the reliability of cesium scintigraphy confirm those of Burguet et al (2) However one apparent drawback of cesium 131 is its low energy which limits the imaging of the heart to the anterior and lateral walls Consequently inferior and posterior wall infarctions are often missed with cesium Furthermore the gamma camera is less suitable for imaging with cesium 131

At present thallium 201 seems to be the radionuclide of choice for myocardial imaging It has a suitable energy spectrum for detection by a gamma camera the physical half life is acceptable and the quality of pictures is usually excellent Uptake of thallium in liver and the gastrointestinal tract may sometimes conceal the lower border of the heart this drawback can be minimized by injecting the isotope after fasting and in the upright position Background uptake is further reduced by exercise The pattern of thallium distribution in the healthy myocardium has also recently been described (4) The results with thallium 201 in patients with previous transmural anterior infarction are similar to those with cesium However with thallium also inferior and posterior infarctions could be visualized Our results in the diagnosis of previous infarction with thallium are similar to those in the diagnosis of acute infarction reported by others (11 13 14) Perfusion defects due to acute infarction may disappear after some time as reported by Wackers et al (13) This fact may explain the failure to diagnose previous infarction in some patients However the same applies to other non invasive methods like ECG

Patients with non informative ECG changes are a suitable target group for scintigraphic investigations Scintigraphic defects were detected in 22 of our 53 patients with non informative ECG changes These defects are probably due to infarction It should however be mentioned that McGowan et al (9) have described for potassium 43 or rubidium 81 false positive scintigraphic defects simulating anteroseptal infarction in patients with LBBB In the remaining patients in this group the result of the scintigraphy excludes a substantial myocardial destruction at least when thallium is used In this context it should be pointed out that decreased uptake is a non specific finding This is

illustrated by the result in the patients with conges-tive cardiomyopathies We were not able to dif-ferentiate reduced uptake by scintigraphy probably due to fibrosis from that caused by coronary heart disease The findings in hypertrophic obstructive cardiomyopathy reported by Bulkley et al (1) were not confirmed in the present study This may be due to technical differences as we used a converging collimator and the American group a parallel hole collimator

The group of patients with angina pectoris de-serves a few comments According to history and ECG no patient had sustained a myocardial infarc-tion In 8 of these 18 patients unequivocal defects were diagnosed scintigraphically It would thus ap-pear that scintigraphy is a valuable diagnostic tool in the evaluation of patients before the decision is made to perform coronary angiography and left ventricular angiography Finally in the group of patients with valvular heart disease scintigraphy is of the same value as in the group with non informative ECG chances Scintigraphy with thal-lium will give information about hypertrophy or dilatation of the left and right ventricle which other non invasive methods such as ECG and roent-genological determination of heart size will not al-ways yield

The accuracy of thallium scintigraphy for the de-tection of previous myocardial infarction has been further documented in a separate series of patients who were all subjected to coronary and left ventric-ular angiography The results of this investigation will be published in a forthcoming paper

ACKNOWLEDGEMENT

Supported by grant no 2872 from the Swedish Medical Research Council

REFERENCES

1 Bulkley B H Rouleau J Strauss H W & Pitt B Idiopathic hypertrophic subaortic stenosis Detection by thallium 201 myocardial perfusion imaging N Engl J Med 293 1113 1975
2 Burguet W Merchie G & Kulbertus H 131Cs myocardial scintigraphy Application to assessment of anterior myocardial infarction Br Heart J 37 1037 1975
3 Carr E A Jr Gleason G Shaw J & Kra(?)z, B. The direct diagnosis of myocardial infarction by photoscanning after administration of cesium-131 Am Heart J 68 627 1964
4 Cook D J Bailey I Strauss H W Rouleau J Wagner H N Jr & Pitt B Thallium-201 for myocardial imaging Appearance of the normal heart J Nucl Med 17 583 1976
5 Cooper M Myocardial imaging—an overview in cardiovascular nuclear medicine (ed H W Strauss, B Pitt & A E James) pp 149–162 Mosby St. Louis 1974
6 Donato L. Bartolomei G & Giordani R. Evalua-tion of myocardial blood perfusion in man with radioactive potassium or rubidium and precordial counting Circulation 29 195 1964
7 Folse R. & Braunwald E Pulmonary vascular dilu-tion curves recorded by external detection in the diagnosis of left to right shunts Br Heart J 24 166, 1962
8 Holman B L. Dewanjee M K Idoine J Fliegel C. P Davis M A Treves S & Eldh P. Detection and localization of experimental myocardial infarction with 99mTc tetracycline J Nucl Med 14 595 1973
9 McGowan R L. Welch T G Zaret B L. Bryson A L. Martin N D & Flamm M D Noninvasive myocardial imaging with potassium-43 and rubidium-81 in patients with left bundle branch block. Am J Cardiol 38 422 1976
10 Parkey R W Bonte F J Meyer S L. Atkins, J M Curry G L Stokely E. M & Willerson, J T. A new method for radionuclide imaging of acute myocardial infarction in humans Circulation 50 540 1974
11 Parkey R W Bonte F J Stokely E. M Lewis, S. E Graham K D Buja, L. M & Willerson J T. Acute myocardial infarction imaged with 99mTc stannous pyrophosphate and 201Tl A clinical evalua-tion J Nucl Med 17 771 1976
12. Roos R S Ueda, K Lichtlen P R & Rees J R. Measurement of myocardial blood flow in animals and man by selective injection of radioactive inert gas into the coronary arteries Circ Res 15 28 1964
13 Wackers F J Th. Busemann Sokole E Samson G v d Shoot J B Lie K I Liem K L & Wellens H J J Value and limitations of thallium-201 in the acute phase of myocardial infarction. N Engl J Med 295 1 1976
14 Wackers F J Th v d Schoot J B Busemann Sokole E Samson G v Niftrik G J C. Durrer D & Wellens H J J Noninvasive visualization of acute myocardial infarction in man with thallium-201 Br Heart J 37 741 1975
15 Zaret B L. Strauss H W Martin N D Wells H P & Flamm M D Non-invasive regional myocar-dial perfusion with radioactive potassium. Study of patients at rest with exercise and during angina pectoris N Engl J Med 288 809 1973

Acta Med Scand 202 357–362 1977

Intravenous Glucose Tolerance Test in Middle-Aged Men with and without Latent Coronary Heart Disease

Jan Erikssen and Sven Chr Enger

From Medical Department B Rikshospitalet Oslo and the Department of Clinical Biochemistry Drammen Sykehus Drammen Norway

ABSTRACT During a cardiovascular survey, aimed at detecting cases of latent coronary heart disease (CHD), glucose elimination was studied after i v loading in 1970 presumably healthy men aged 40–59 years The aim was to throw light on the importance of deranged glucose tolerance for the development of CHD Of the 1970 individuals 1798 were defined as ' normals' 33 had chronic, non anginal chest pain, 34 had slight albeit typical angina pectoris The remaining 105 had various symptoms/signs strongly suggestive of CHD, and underwent diagnostic coronary angiography (69 angiopositive 36 angionegative) Plasma insulin was determined in relation to the test in 249 of the subjects The following conclusions were reached 1) Mean *k* values were similar in all subgroups ($p>0$ 10) 2) Low and borderline *k* values were significantly more frequent in angiographed individuals compared with the group of normals ($p<0$ 025) However an almost identical frequency was seen in angiopositive and angionegative cases 3) *k* values did not change with age between 40 and 59 years 4) *k* values were unrelated to the severity of angiographic findings in individuals with proven CHD 5) Significantly lower *k* values were found in individuals with a positive diabetic heredity, and 6) in individuals with a high insulin response 7) The i v glucose loading did not influence an exercise ECG recorded in relation to a near maximal bicycle exercise test

Both retrospective and prospective studies have pinpointed diabetes mellitus and lesser derangements of glucose metabolism as risk factors for future coronary heart disease (CHD) (2 3 6 7 14 15 18 22 25 26 33 34)

As part of a cardiovascular survey with the primary intent of disclosing latent CHD in apparently healthy middle aged men glucose tolerance has been evaluated by means of an i v glucose load (IVGTT)

The particular aims of the present study were 1) To assess the prevalence of pathologic IVGTTs in five clinical groups of middle aged men previously thought to be healthy 2) To evaluate whether the IVGTT could discriminate *a)* between individuals with and without symptoms/signs of latent CHD according to the preselected survey criteria and *b)* between individuals with true and false positive latent CHD as judged by coronary angiography 3) To find out if the results of the IVGTT are dependent on age 4) To judge the possible relations between the IVGTT and various biochemical and physiological parameters claimed to be risk factors for CHD

STUDY POPULATION

All men aged 40–59 years from five major companies/governmental institutions in Oslo Norway were invited to participate in the study provided they were free from known CHD other known heart disease hypertension under treatment with drugs diabetes mellitus malignancy disorders of the locomotor system preventing a near maximal bicycle exercise test and miscellaneous diseases The detailed selection procedures are presented elsewhere (9 11) In total 2014 men (86% of the eligible men) met for the examination which started at 07 30 a m after at least 12 hours fasting

The examination included a comprehensive case history clinical examination height/weight measurements lung function tests X ray of the heart/lungs phonocardiography resting ECG a near maximal bicycle exercise test and several biochemical tests including cholesterol triglycerides lipid electrophoresis in selected cases fasting blood glucose and an IVGTT All individuals had a complete examination program

A diabetic heredity was said to be present if diabetes had been diagnosed in parents siblings or children

Table I *Distribution of k values among 1970 apparently healthy middle aged men*

λ value	Group I (n=1798)	Group II (n=33)	Group III (n=34)	Group IV (n=36)	Group V (n=69)	Total (n=1970)
<1.10						
n	241 (80)	3 (1)	4 (2)	9 (4)	16 (8)	273 (95)
%	13.4	9.1	11.8	25.0	23.2	13.86
≥1.10						
n	1 557	30	30	27	53	1 697
%	86.6	90.9	88.2	75.0	76.8	86.14
Mean	1.93	1.81	1.80	2.07	1.76	1.92
S.D.	0.90	0.73	0.90	1.13	0.81	

Figures within parentheses indicate cases with diabetic λ values (<0.90)
χ^2 for the distribution of λ values of ≥1.10 and <1.10 for all five groups=8.63 (d.f.=4, p=0.07), of λ values of <0.90, 0.90–1.09 and ≥1.10=12.53 (d.f.=8, p=0.13) and of λ values of ≥1.10 and <1.10 for groups I–IV and V=9.01 (d.f.=?, p<0.025)
ᵃ No significant differences among groups (p>0.10 for all comparisons)

A diagnosis of previously undiagnosed CHD was made in the presence of any of the following criteria: 1) A positive WHO questionnaire (WHO Q) on angina pectoris on personal interview (29). 2) A positive exercise ECG during and/or after exercise (9, 11). 3) Angina pectoris occurring during the exercise test. 4) Presence of Minnesota Code 1.1 in the resting ECG ("very probable infarction pattern") (29). 5) A positive Greater New York Health Insurance Plan Survey questionnaire (NY Q) on angina pectoris (12).

In all the 115 men who met any of the criteria 1–4 the suspicion of CHD was thought to be sufficiently strong to warrant coronary angiography in individuals who gave their informed consent (9). Individuals who had a positive criterion 5 only were not offered coronary angiography.

Exclusions. Of the 115 men who were offered coronary angiography, 6 refused and 109 gave their informed consent, 4 of whom were later excluded for various reasons (9). Three of the refusers had angina pectoris and entered group III (see below). The other 3 refusers were excluded. In addition, 37 men were excluded for one or more of the following reasons: 1) Extravasal injection of glucose. 2) Severe vasovagal reaction. 3) Physical activity during the test. Thus 1970 individuals were left for this particular study.

The 1970 individuals were grouped according to the clinical and angiographical criteria as follows: *Group I*: Normals, n=1798. *Group II* (atypical chest pain): Thirty three individuals with chronic chest pain answering "yes" to the first two questions of the WHO Q but with a negative NY Q. *Group III* (angina pectoris, not angiographed): Thirty four individuals with angina pectoris according to NY Q but with no other signs of latent CHD (see above). *Group IV* (angionegative): n=36 (Selection criteria for angiography, see above.) *Group V* (angiopositive): n=69.

METHODS

An i.v. injection of 25 g glucose (50 ml 50% solution w/v) was administered during 2–3 min with the subjects in semiprone position. Zero time was defined as the end of the injection. Blood glucose was determined according to Hjelm and de Verdier (16) before glucose injection, at time zero and thereafter every 10 min in the first 800 individuals and every 15 min in the remainder. The values from 10 or 15 min on were plotted on a semilogarithmic scale for assessment of the k value. If two glucose values during the test were ≥10 mg/100 ml below the fasting value, only the first was used for determining the k value. In 50 men the k value was also assessed by regression analysis of the logarithmic glucose values. As these k values obtained mathematically did not differ significantly from those obtained directly from the semilogarithmic plots, the latter were accepted for this presentation. λ values of ≥1.10 were defined as normal, below 0.90 as diabetic and within the range of 0.90–1.09 as borderline.

In 249 individuals immunoreactive insulin was determined in samples taken before and 15, 30, 45 and 60 min after the glucose loading. The mean of these five insulin values was calculated in each subject. High and low insulin responses were defined arbitrarily as follows: *High insulin response*: Mean insulin >32 μU/ml, or mean >25 *and* at least two of the following: fasting value >25, 30-min value >30, 45 min value >30, 60-min value >25. The arbitrary value of 32 μU/ml as a definition of a high insulin response was chosen as 2 S.D. above the mean of mean insulin values found in 62 individuals with reference body

Table II. *λ values in relation to age among 1798 apparently healthy men*

	Age (y.)				
	40–44	45–49	50–54	55–59	Total
No. of men	377	543	487	391	1 798
K value	1.99	1.93	1.90	1.91	1.93

One way analysis of variance indicates a p value of >0.10 for all group comparisons and=0.42 for equality of group means.

Table III *Correlation coefficients between k values and various clinical/physiological parameters among 1970 apparently healthy middle aged men*

Group	BMI[a]	B wt	BP Systolic	BP Diastolic	Cholesterol	Fasting triglycerides	Fasting blood glucose
I (n=1 798)	−0 145***	−0 145***	−0 224***	−0 189***	−0 043	−0 060*	−0 357***
II (n=33)	−0 113	−0 163	−0 102	−0 075	−0 063	+0 274	−0 106
III (n=34)	−0 113	−0 050	−0 362*	−0 438*	−0 301	−0 030	−0 286
IV (n=36)	+0 028	−0 057	−0 216	−0 240	−0 122	−0 257	−0 434**
V (n 69)	−0 276*	−0 338**	−0 155	−0 105	+0 106	−0 234	−0 353***

[a] Body mass index b wt (kg)/b ht (cm)−100
*p<0 05 **p<0 01 ***p<0 001

weight and normal glucose tolerance tests lipids and BP *Low insulin response* Mean insulin level <5 μU/ml *or* fasting and 15 min values <5 μU/ml Insulin was assayed according to Wide and Porath (35) in duplicate as immunoreactive insulin in heparinized plasma (Phadebas Insulin Test Pharmacia)

The correlation coefficients (r values) were assessed between k values and the following parameters body mass index (body weight (kg) divided by (height (cm)−100)) total body weight systolic and diastolic BP *cholesterol* triglycerides and fasting blood glucose In addition mean k values were assessed in relation to the detailed coronary angiographic findings among the 105 angiographed individuals (with normal angiograms one two- and three vessel disease)

Tests of significance used were χ^2 test multiple t tests one way analysis of variance and linear correlation test (4)

RESULTS

Of the 1 798 normals 241 (13 4%) had a k value of <1 10 and 1 557 (86 6%) ≥1 10 A diabetic k value i e <0 90 was found in 80 subjects (4 5%) (Table I) Groups IV and V had approximately twice as high a frequency of borderline and diabetic values as group I

However this difference in distribution does not reach statistical significance when all five groups are considered (χ^2=8 63 d f =4 p=0 07) If only groups I IV and V are considered (i e normals vs the two groups of angiographed individuals) χ^2 for the distribution of k values below and above 1 10=9 01 (d f =2 p<0 025) There are no significant differences in mean k values between any groups

No age trend in mean or extreme k values was found (Table II)

Several of the correlation coefficients presented in Table III reach a high statistical significance although the associations are weak Thus for instance only 13% of the variance of the k values is explained by variation in fasting glucose the parameter which showed the strongest association with the k values No association was found between k values and cholesterol and only a negligible association between fasting triglycerides and k values

Individuals with a positive diabetic heredity had a lower k value than those with a negative heredity (p<0 001) although the absolute difference in mean values is small (Table IV)

Table IV *K values in relation to heredity of diabetes among 1798 apparently healthy middle aged men*

	Diabetic heredity Positive	Diabetic heredity Negative	Total
N	235	1 563	1 798
%	13 0	87 0	
Mean k value	1 70	1 97	1 93

One way analysis of variance indicates a p value of <0 001 for the difference in mean values between individuals with a positive vs a negative heredity of diabetes

Table V *K values and log k values in relation to insulin response during IVGTT in 249 apparently healthy middle aged men*

	Insulin response 1 High (n=36)	2 Normal (n=195)	3 Low (n=18)	Mean
K values[a]	1 44	2 31	1 83	2 15
Log k values[b]	0 142	0 309	0 212	0 272

Levels of significance according to one way analysis of variance
[a] $p_{1\ 2}$<0 001 $p_{1\ 3}$=0 039 $p_{2\ 3}$=0 10
[b] $p_{1\ 2}$=0 001 $p_{1\ 3}$=0 13 p_{2-3}=0 067

Table VI *K values and fasting blood glucose in relation to detailed coronary angiographic findings among 105 men with a preangiographic diagnosis of suspected CHD*

	Normal coronary angiograms	One vessel disease	Two-vessel disease	Three vessel disease
No of men	36	16	25	26
K values*				
Mean	2 07	1 47	1 94	1 77
S D	1 13	0 73	0 82	0 78
Fasting blood glucose (mmol/l)				
Mean	4 61	4 78	4 59	4 49
S D	0 58	0 58	0 83	0 53

* $p>0.10$ for all comparisons among groups

Individuals with a normal insulin response had the highest and individuals with a high insulin response the lowest *K* values (Table V) The difference in mean *K* values between individuals with a normal and a high insulin response was highly significant ($p<0.001$) between individuals with low and normal response significant at a lower level and between high and low insulin responders insignificant ($p>0.10$)

The mean *K* values are presented in Table VI according to the detailed angiographic findings In dividuals with normal angiography had the highest *K* values although the overall differences between angiopositive and angionegative individuals is insignificant ($p>0.10$) Neither is there any trend in *K* values in relation to the extent of coronary pathology (one vs two- or three vessel disease)

During the run in period of the survey we demonstrated that the IVGTT did not influence the exercise ECG as judged by a number of repeated exercise tests performed within a fortnight of the first one These presurvey findings were amplified by similar findings during the survey and in particular no influence was seen in individuals with a positive exercise ECG all of whom had repeated exercise tests

DISCUSSION

The IVGTT is a recognized and much used tool for evaluating glucose tolerance (20 30 33 34) Wahlberg (33) reports adequate reproducibility of the test in CHD patients However recently Hedstrand and Boberg (13) demonstrated a poor reproducibility of the IVGTT in healthy middle aged male volunteers It is claimed that the oral glucose tolerance test (OGTT) is more reliable in detecting decreased glucose tolerance than the IVGTT (13 17 23) although a poor repeatability has been reported for the OGTT as well (19)

As exercise ECG testing was one of the main objectives of the study OGTT could not be used since ingestion of any kind of food may influence the exercise ECG in an unpredictable way The main reason for choosing the IVGTT in the present study was that it did not influence the exercise ECG in repeated tests during the run in period These findings were confirmed during the survey proper

Borderline or diabetic *K* values were found in 13% of normals (group I) which compares well with Wahlberg s findings in normal controls (33) The prevalence (approximately 25%) of borderline/diabetic *K* values among our CHD suspect individuals was however far below the 60% which Wahlberg reports as the mean prevalence in patients with CHD from the literature (34)

The mean *K* values in our normals compare well with some reports (5 20 22) but are somewhat higher than in some others (13, 30 33 34) The exclusion of diabetics and individuals using diuretics should tend to increase the mean *K* values as does the exclusion of individuals with vasovagal reactions However recalculation of the *K* values with the latter individuals included changes them very little

Different studies have used different glucose loads (5, 13 20 22) and the influence of this must be a matter for speculation although the fixed dose of 25 g glucose should make our results comparable with Wahlberg s (33)

However the *K* values in our subjects with proven CHD were far higher than those reported in patients with clinical CHD and peripheral atheromatosis (33 34) This discrepancy is unrelated to the severity of the angiographic findings since similar *K* values were noted in individuals with one two and three vessel disease

The reasons for the discrepancy between the *K* values in our CHD subjects and CHD subjects in the literature are obscure However retrospective studies deal with diseased subjects i e individuals who have sought medical advice Therefore a tentative (although purely speculative) hypothesis to explain the low prevalence of glucose intolerance in our CHD subjects compared with findings in clini

cal CHD might be that when coronary atheromatosis is present complications (i e angina/MI) are more likely to occur in individuals with the most severe glucose tolerance aberration It has for instance been shown that platelet adhesiveness is increased in diabetics (21) which makes diabetics more prone to arterial thrombosis whereas we have found both platelet aggregation and adhesiveness to be unrelated to the k value and fasting glucose in a subgroup of 490 individuals from our series (10)

Inevitably a study like ours misclassifies several individuals as normals when in fact they have advanced coronary artery disease Follow up should diminish this misclassification in our study and might possibly lead to better agreement with data from the literature However the misclassification in our study should be far less than in studies in which the investigators were unable to validate their CHD suspect diagnoses by means of coronary angiography Thus we were able to show that approximately 1/3 of our CHD suspect individuals were free from significant CHD (9)

In a study like ours—which is classified by Olsson and Carlson (24) as interspective—one picks up preclinical CHD cases and the findings therefore may reflect associations during earlier stages of the disease That the majority of these subjects were true preclinical is reflected by the fact that the great majority were suspected solely from a positive exercise test (9)

While the literature is almost monotonous in its conformity concerning the strong positive relation between clinical CHD and glucose intolerance (2 3 6 7 14 15 18 22 25 26 33 34) we have found little difference between our CHD free and CHD suspect subjects as have Rifkind (28) and Ryan et al (30) Thus the IVGTT only marginally distinguished between healthy and CHD-suspect subjects However the test did not discriminate between true and falsely suspect CHD subjects

It has been said that glucose tolerance deteriorates with age although there is no general agreement about this in individuals below 60–70 (33 34) No age trend was found in our study which may be due to our strict age limits Thus the weak negative association between k values and age reported by Wahlberg (33) came from the subclass of individuals above 70 years

The weak or non existent associations between k values and the various clinical and biochemical parameters are well in line with previous experience (1 6 27 33) and add support to the concept that aberrations of glucose metabolism act as an independent risk factor for CHD (6 7 18 25)

In discordance with some others (33 34) we have found a significantly lower k value in individuals with a positive diabetes heredity

REFERENCES

1 Carlson L A & Wahlberg F Serum lipids in travenous glucose tolerance and their interrelation studied in ischaemic cardiovascular disease Acta Med Scand 180 307 1966
2 Christiansen I Deckert T Kierulf K Midtgaard K & Worning H Glucose tolerance plasma lipids and serum insulin in patients with ischaemic heart diseases Acta Med Scand 184 283 1968
3 Cohen L S Elliott W C Klein M D & Gorlin R Coronary heart disease Clinical cinearteriographic and metabolic correlations Am J Cardiol 17 153 1966
4 Dixon W J (Editor) Biomedical computer programs (Revision 1975) Developed at the Health Sciences Computing Facility UCLA sponsored by NIH Special Research Grant RR 3 University of California Press Berkeley 1975
5 Enger S Chr & Ritland S Glucose tolerance insulin release and lipoprotein pattern in patients after myocardial infarction Acta Med Scand 194 97 1973
6 Epstein F H Hyperglycemia A risk factor in coronary heart disease Circulation 36 609 1967
7 — Epidemiological studies of cardiovascular disease in a total community—Tecumseh Michigan Ann Intern Med 62 1170 1965
8 Eriksson J Unpublished data
9 Erikssen J Enge I Forfang K & Storstein O False diagnostic tests and coronary angiographic findings in presumably healthy middle aged men Circulation 54 371 1976
10 Erikssen J Hellem A & Stormorken H Platelet adhesiveness in presumably healthy middle aged men To be published
11 Erikssen J Rasmussen K Forfang H & Storstein O Exercise ECG and case history in the diagnosis of latent coronary heart disease among presumably healthy middle aged men Eur J Cardiol In press 1977
12 Frank C W Weinblatt E & Shapiro S Angina pectoris in men Prognostic significance of selected medical factors Circulation 47 509 1973
13 Hedstrand H & Boberg J Statistical analysis of the reproducibility of the intravenous glucose tolerance test and serum insulin response to this test in middle aged men Scand J Clin Lab Invest 35 331 1975
14 Heinle R A Levy R I Fredrickson D S & Gorlin R Lipid and carbohydrate abnormalities in patients with angiographically documented coronary artery disease Am J Cardiol 24 178 1969
15 Herman M V & Gorlin R Premature coronary artery disease and the preclinical diabetic state (Editorial) Am J Med 38 481 1965

16 Hjelm M & de Verdier C H A methodological study of the enzymatic determination of glucose in blood Scand J Clin Lab Invest 15 415 1963

17 Jackson R L Guthrie R A Murthy D Y N & Lang J The intravenous glucose tolerance test in chemical diabetes Metabolism 22 247 1973

18 Keen H Rose G Pyke D A Boyns D Chlouverakis C & Misty S Blood sugar and arterial disease Lancet 2 505 1967

19 Kingsbury K J Glucose tolerance age and atherosclerosis Postgrad Med J (Suppl) 44 944 1968

20 Lundbæk K Intravenous glucose tolerance as a tool in definition and diagnosis of diabetes mellitus Br Med J 1 1507 1962

21 Mayne E Bridges J H & Weaver J A Platelet adhesiveness plasma fibrinogen and factor VIII level in diabetes mellitus Diabetologia 6 436 1970

22 Nikkilä E A Miettinen T A Vessenne M R & Pelkonen R Plasma insulin in coronary heart disease Lancet 2 508 1965

23 Olefsky J M Farquhar J W & Reaven G M Do the oral and intravenous glucose tolerance tests provide similar diagnostic information in patients with chemical diabetes mellitus? Diabetes 22 202 1973

24 Olsson A G & Carlson L A Studies in asymptomatic primary hyperlipidemia I Types of hyperlipoproteinemias and serum lipoprotein concentrations compositions and interrelations Acta Med Scand (Suppl) 580 1975

25 Ostrander L D Jr Francis T Hayner N S Kjelsberg M O & Epstein F H Relationship of cardiovascular disease to hyperglycemia Ann Intern Med 62 1188 1965

26 Paasikivi J Long term tolbutamide treatment after myocardial infarction Acta Med Scand (Suppl) 507 1970

27 Reaven G Calciano A Cody R Lucas C & Miller R Carbohydrate intolerance and hyperlipemia in patients with myocardial infarction without known diabetes mellitus J Clin Endocrinol Metab 23 1013 1963

28 Rifkind B M Plasma free fatty acid levels and intravenous glucose tolerance after myocardial infarction J Atheroscler Res 6 26 1966

29 Rose G & Blackburn H Cardiovascular survey methods WHO Monogr Ser 56 1968

30 Ryan W G Economou P G & Schwartz T B Intravenous glucose tolerance test in an industrial population Controls subjects with coronary heart disease and relatives of diabetics Diabetes 16 171 1967

31 Sievers J Myocardial infarction Clinical features and outcome in three thousand thirty six cases Acta Med Scand (Suppl) 406 1963

32 Sowton E Cardiac infarction and the glucose tolerance test Br Med J 1 84 1962

33 Wahlberg F Intravenous glucose tolerance in myocardial infarction angina pectoris and intermittent claudication Acta Med Scand (Suppl) 453 1966

34 Wahlberg F & Thomasson B Glucose tolerance in ischaemic cardiovascular disease In Carbohydrate metabolism and its disorders (ed F Dickens) vol 2 p 182 Academic Press London 1968

35 Wide L & Porath J Radioimmunoassay of proteins with the use of sephadex-coupled antibodies Biochim Biophys Acta 130 257 1966

Acta Med Scand 202 363–366 1977

Preliminary Observations on Interventricular Septum Vibrations

An Echocardiographic Sign in Aortic Valvular Stenosis

PRELIMINARY REPORT

M Vukas I Wallentin and Å C Hjalmarson

From the Departments of Thoracic Surgery Clinical Physiology I and Medicine I Sahlgren s Hospital University of Goteborg Goteborg Sweden

ABSTRACT In previous experimental and clinical studies on aortic valvular stenosis it has been suggest ed that in addition to the degree of stenosis other fac tors such as the pathoanatomy of the valve and tur bulence induced vibrations might be of importance Using echocardiography, vibrations of the inter ventricular septum were demonstrated in six con secutive patients with congenital aortic stenosis In comparable normal subjects no such vibrations could be seen The clinical significance of ventricular wall vibrations has not yet been established

In a retrospective study of patients operated on for congenital aortic stenosis a poor correlation was found between the pressure gradient of the valve and the symptoms and prognoses (4) It was there fore postulated that other factors such as the pathoanatomy of the aortic valve and turbulence induced vibrations of the ventricular wall might be of importance In animal experiments it was found that a peripheral location of the stenotic opening in the aortic valve more markedly depressed heart function compared with stenosis with central open ing (6) Using the isolated rabbit papillary muscle and the working rabbit heart preparation it was found that mechanically induced vibrations had a myocardial depressant effect (5 7)

The present study was undertaken to investigate whether ventricular wall vibrations could be dem onstrated by echocardiography in patients with con genital aortic valvular stenosis The aim of this pre liminary study was merely to investigate the pos sibility of detecting and quantifying such vibrations Six consecutive patients with congenital aortic valvular stenosis were compared with four healthy individuals of similar ages

STUDY POPULATION AND METHODS

Six patients with congenital aortic valvular stenosis aged 10–30 years were investigated and compared with four healthy subjects aged 7 9 33 and 35 years All four con trols were well known to the authors and they had never shown any symptoms or signs of heart disease Subjective and objective variables from the six patients are presented in Tables I and II

The patients had been admitted to the Department of Clinical Physiology for assessment of aortic valvular dis ease with non invasive techniques In five patients cath-eterizations were previously performed according to methods used routinely in the Departments of Paediatrics and Medicine Data from the invasive investigation were obtained from these records The degree of aortic valvu lar stenosis was judged from angiographic recordings Echocardiography was performed using an Echoscan 30 Ultrasonoscope (Ekoscann AB) with a 2 25 MHz trans ducer with 10 cm focus and with a repetition rate of 1 000 impulses/sec The records were obtained with a strip chart recorder (Honeywell no 1856 Fiber Optic Recorder) The standard technique for examining the left ventricle was followed (2) Much care and time were taken to get the antero-posterior diameter of the left ventricle just below the aortic root and through or very close to both the an terior and the posterior mitral leaflets With this position of the transducer the septum was extremely enlarged and its movements were registered at high speeds (around 20 cm/sec) The stroke volume was calculated from the end diastolic and end systolic volumes derived by the cube method (3) and the ejection fraction from the stroke volume and end diastolic volume The mean rate of cir cumferential fibre shortening (mean V_{cf}) was calculated according to Cooper et al (1) with the exception that the left ventricular ejection time in the formula was derived from the carotid pulse curve

RESULTS

In six patients with congenital aortic stenosis rou tinely followed up for several years using various

Table I *Characterization of patients with aortic valvular disease from symptoms heart size and invasive measurements*

Pat no	Age (y)	Symptoms	Functional class[a]	Heart size at X ray[b] (ml/m² BSA)	Aortic peak systolic gradient[c] (mmHg)	Angiographic aortic regurgitation
1	10	None	I	350/350	60–70	None
2	11	None	I	350/320	120	None
3	12	Mild	I	420/420	120	None
4	13	Mild	II	460/360	25–30	Moderate
5	24	None	I	710/400	NM	NM
6	30	Moderate	III	–/520	90	Moderate

[a] Stated according to the New York Heart Association classification (1974)
[b] Radiological estimation of heart size has been done with the patient in the standing position
[c] Estimated by catheterization with simultaneous recording
NM = not measured

clinical methods echocardiographic pictures were taken of enlarged interventricular septum in order to register vibrations The data from the invasive and non invasive investigations are summarized in Tables I and II As judged from the peak systolic gradient (30–130 mmHg) the patients ranged from mild to severe aortic valvular stenosis Non invasive investigations showed typical signs of moderate to severe valvular aortic stenosis in all cases and in three there were also signs of moderate regurgitation which was confirmed in two cases by angiography No patient had signs of left ventricular failure The mean velocity of circumferential fibre shortening and the ejection fraction were within the normal range in the six patients with aortic valvular disease The echocardiographic values of stroke volume and cardiac output were high in patients 4 5 and 6 due to regurgitation

In all six patients with congenital aortic valvular stenosis clear oscillations were registered both in the septum and in the anterior wall of the left ventricle (Fig 1 Table II) The amplitude of the vibrations varied from case to case and also from one cardiac cycle to another in one and the same patient The maximum amplitude was located around the middle of systole and ranged from 1 2 to 2 5 mm The frequency of the vibrations varied between 125 and 180 oscillations/sec The values in the tables are from three consecutive cardiac cycles Four healthy age matched subjects were studied echocardiographically and no significant vibrations of the septum or the anterior wall could be detected

DISCUSSION

A pressure gradient over the stenotic aortic valve is widely used as a guide to the severity of the stenosis and for planning heart surgery In a retrospective

Table II *Non invasive parameters in patients with aortic valvular disease*

		Septal vibrations					Estimated echocardiographic parameters			
							Including the regurgitations			
Pat no	Age (y)	Amplitude (mm)	Frequency (oscill/sec)	LVED diameter (cm)	Mean V_{cr} (circumfer/sec)	Ejection fraction	Stroke volume	Cardiac output (l/min)	Aortic peak systolic gradient (mmHg)	Aortic regurgitation
1	10	1 3–1 6	94–106	3 6	1 59	0 83	39	3 5	40–50	None
2	11	1 3–1 5	110	3 5	1 55	0 80	35	3 0	50–80	None
3	12	2 1–2 4	107–128	3 8	1 43	0 85	46	3 6	100–130	None
4	13	1 8–2 2	91–106	6 7	1 20	0 80	240	20	30	Moderate
5	24	1 2–1 4	100–110	5 1	1 13	0 75	105	6 6	<30	Mild
6	30	2 1–2 5	148–180	5 2	1 15	0 79	111	8 7	100	Moderate

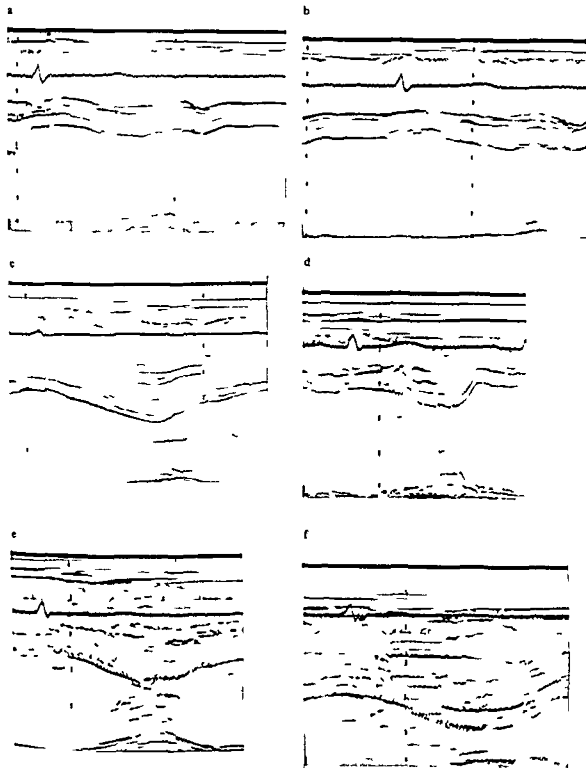


Fig. 1. Septal vibrations from echocardiography in controls and patients with aortic valvular disease. (*a*) Normal female aged 8 years. (*b*) normal female aged 9 years. (*c*) normal female aged 34 years. (*d*) patient 2, male aged 11 years. (*e*) patient 3, male aged 12 years. (*f*) patient 4, male aged 12 years.

study of patients operated on for congenital aortic stenosis a poor correlation was found between the pressure gradient and the presence of symptoms (2) It was postulated that other factors might be of importance for cardiac function Such factors might be the pathoanatomy of the stenotic valve and the presence of turbulence induced vibrations of the ventricular wall In an isolated rabbit heart preparation it was found that a peripheral location of the stenotic opening in the aortic valve depressed heart function more markedly than a central stenotic opening (6) In the study of 32 patients with congenital aortic stenosis the location of the remaining opening of the stenotic aortic valve did not seem to be related to the symptoms (2) Since vibrations of the myocardium in experimental models showed depressed function (5 7) it is possible that turbulence induced vibrations influence heart function also in man

The question arose whether it is possible to register and quantify vibrations of the ventricular walls in the human heart It is well known that vibrations of the different valves can be registered by echocardiography In aortic regurgitation the detection of diastolic vibrations of the mitral leaflets and of the septum is a routine procedure To our knowledge however systolic vibrations have not been reported in aortic stenosis Using echocardiography in the present study vibrations of the interventricular septum were demonstrated in six consecutive patients with congenital aortic stenosis In normal subjects of comparable age and sex no such vibrations could be seen These preliminary findings show that echocardiography may be used for detection of vibrations and that this is a pathological sign The importance of these vibrations remains to be proved Prospective studies have to be performed with correlation of the amplitude and frequency of vibrations with the pathoanatomy of the aortic valve in patients subjected to surgery

ACKNOWLEDGEMENTS

This investigation was supported by grants from the Swedish Medical Research Council (B76-19X-0?5?9 17A) the Swedish National Association against Heart and Chest Diseases the Faculty of Medicine University of Göteborg Göteborg Medical Society and AB Hässle

REFERENCES

1 Cooper R H O Rourke R A Karliner I S Peterson K L & Leopold G R Comparison of ultrasound and cineangiographic measurements of the mean rate of circumferential fiber shortening in man Circulation 46 914 1972
2 Feigenbaum H Echocardiography Lea & Febiger Philadelphia 1972
3 Pombo I F Troy B L & Russell R O Left ventricular volumes and ejection fraction by echocardiography Circulation 43 480 1971
4 Vukas M Carlgren L E & Swedberg J Congenital aortic stenosis A retrospective study on 3? operated patients Acta Med Scand To be published
5 Vukas M Málek I & Hjalmarson Å C Myocardial depressant effect of vibrations in the isolated rabbit heart Scand J Clin Lab Invest Submitted for publication 1977
6 Vukas M Málek I Hjalmarson Å C & Swedberg J The importance of the pathoanatomy of the aortic stenosis A study in an isolated working rabbit heart preparation Scand J Clin Lab Invest Submitted for publication 1977
7 Vukas M Sivertsson R & Ljung B Inhibitory effects of vibrations on contractility of isolated rabbit papillary muscle Scand J Clin Lab Invest Submitted for publication 1977

Acta Med Scand 202 367–372 1977

Comparison of Postoperative Coumarin, Dextran 40 and Subcutaneous Heparin in the Prevention of Postoperative Deep Vein Thrombosis

F van Geloven P Wittebol and J J Sixma

From the Departments of Surgery and Haematology University Hospital Utrecht The Netherlands

ABSTRACT A double blind study was carried out to investigate the effectiveness of several preventive regimens in postoperative deep vein thrombosis (DVT) The regimens consisted of postoperative (p o) acenocoumarin, dextran 40 + p o acenocoumarin, subcutaneous (s c) heparin alone and s c heparin + p o acenocoumarin The 313 patients studied were *stratified according to age (40–60 vs >60* years) and type of operation (laparotomy, thoracotomy, hip replacement) Dextran 40+p o acenocoumarin was more effective than p o acenocoumarin alone, which acted as control Subcutaneous heparin alone or together with p o acenocoumarin was not more effective than p o acenocoumarin alone during the first part of the study, when about 4000 IU twice daily were administered accidentally When the dose had been changed to 5000 IU twice daily, better results were obtained (DVT incidence 5 9%) The results were strongly influenced by age and type of operation Almost no DVT occurred in patients below 60 years of age with elective abdominal surgery The incidence of perfusion disturbances in lung scans in patients with DVT was lowest in those treated with s c heparin in combination with acenocoumarin

In recent years considerable progress has been made in the detection of subclinical postoperative (p o) deep vein thrombosis (DVT) and its prevention Most studies have shown that accumulation of radioactive labelled fibrinogen as detected by scanning of the legs offers a reliable prediction of DVT (1 3 17 19) Subcutaneous (s c) heparin 5000 IU two or three times daily has been shown to be effective in the prevention of p o DVT and pulmonary embolism (4 5 8 9 18) Dextran 70 and 40 were shown to prevent DVT as judged from studies in which the diagnosis was based on clinical criteria (6 11) but its effectiveness in preventing subclinical DVT is disputed (6 10 12 14 15 16) Postoperative coumarins do not decrease the frequency of subclinical DVT (13 19) in contrast to preoperatively started coumarins which are very effective (13)

The investigation which we report here has a number of special characteristics It was devised as a comparison between p o coumarins (control) dextran 40 + p o coumarins s c heparin alone or in combination with p o coumarins The study was designed as a double blind investigation and the patients *were stratified according to age and type of* operation The study fell short of its aim when too low a dose of heparin (about 4000 IU twice daily) was accidentally administered during the first part but this allowed us to shed some light on the critical dose level of s c heparin therapy

PATIENTS AND METHODS

Patients

In principle all patients from the Department of General Surgery were admitted to the study if they met the following criteria Over 40 years of age and undergoing elective *laparotomy thoracotomy or hip replacement duration of* anaesthesia over one hour no contraindications Contraindications were (*a*) preoperative use of coumarins (*b*) a thromboembolic process less than 3 months previously (*c*) a bleeding tendency (*d*) contraindications for any of the drugs utilized or iodine (*e*) very serious varices of the leg which might interfere with the ^{125}I fibrinogen scanning

The patients were stratified according to age (40–60 vs over 60 years of age) and type of operation (laparotomy

Table I *Incidence of DVT in the four treatment groups*

A=p o acenocoumarin B=dextran 40+p o acenocoumarin C=s c heparin 4000 IU twice daily D=p o acenocoumarin + s c heparin 4000 IU twice daily

DVT	A	B	C	D
Present	20	9	15	13
%	25	11 4	18 7	17 6
Absent	60	70	65	61
Total	80	79	80	74

A vs B χ^2=4 94 p=0 05 χ^2 test with Yates correction for continuity

vs thoracotomy vs hip operation) Patients admitted to any of the six classes were randomly allocated to one of the four regimens

Data recorded

Preoperative assessment Name age height weight and sex were recorded Hb haematocrit and total serum protein were assayed Also recorded were the type of operation presence of malignancy diabetes mellitus and varices of the legs

Peroperative data The blood loss type and duration of anaesthesia and operation and the fluids administered were recorded Blood loss was estimated by weighing the wound dressings and assessing the blood obtained by suction

Postoperative Hb haematocrit and total serum protein were assayed at two day intervals The thrombotest clotting time to determine the degree of anticoagulation was estimated daily but the results were kept in the laboratory A ^{125}I labelled fibrinogen scan was made daily during the first 10 p o days A clinical examination for DVT and pulmonary embolism was carried out daily by one of us (F G) All patients with a positive ^{125}I labelled fibrinogen scan were subjected to a chest X ray and pulmonary perfusion scintigraphy with ^{99m}Tc labelled macroaggregates of human albumin

Treatment schedules

Four treatment regimens were utilized

A *Postoperative acenocoumarin* (Sintrom) This treatment consisted of the administration of acenocoumarin as indicated by the consultant in internal medicine who was aware of the treatment regimen and received the thrombotest clotting times from the laboratory The treatment was started on the first p o day The patients also received a placebo dextran infusion during the operation and 24 hours later on the first p o day and were injected twice daily in the abdominal skin with placebo heparin starting two hours before the operation

B *Dextran 40 and p o acenocoumarin* The treatment was similar to A but these patients received 500 ml dextran 40 during the operation and 500 ml 24 hours later on the first p o day

C *Subcutaneous heparin alone* The regimen was similar to A Placebo acenocoumarin was also changed in dosage by the internist although no clotting prolongation was of course observed Placebo heparin was replaced by true heparin Placebo dextran 40 was infused

D *Subcutaneous heparin and p o acenocoumarin* Acenocoumarin was administered True heparin was administered during 4½ days and then replaced by placebo (see protocol) Placebo dextran was infused too

Protocol of trial

Random allocation of patients occurred in groups of 20 making it possible to stop the trial at any desired moment A table of random numbers was used to obtain a fortuitous sequence of the numbers from 1 to 20 Having been admitted to the trial a patient was given the next number in the respective subclass The number corresponded to a treatment package prepared by the hospital pharmacy containing two 500 ml bottles of dextran 40 (Rheomacrodex 10% in 2 volumes glucose 5% to 1 volume saline) or two bottles of glucose/saline alone one package containing 30 tablets with 4 mg acenocoumarin or 30 placebo tablets (containing saccharum lactis) one package marked heparin I containing 9 ampoules of calcium heparin (5000 IU in 0 2 ml) or saline and another package marked heparin II containing 10 ampoules of either calcium heparin (5000 IU in 0 2 ml) or saline The heparin was divided into two different lots because its administration was stopped after 4 days in regimen D The trial was blind and a second package was necessary which contained saline in the case of regimen D and heparin in the case of regimen C

RESULTS

Of the 313 patients participating in the trial 80 received treatment A 79 treatment B 80 treatment C and 74 treatment D The patients age sex and body weight were similar in the four treatment groups The duration of anaesthesia and operation and the incidence of malignancy were also similar A further 18 patients were admitted to the trial but not studied Of these seven died within the first

Table II *Results of treatment with various regimens*

Treatment groups as in Table I

Age group	Thoracotomy 40–60	Thoracotomy 60	Laparotomy 40–60	Laparotomy 60	Hip replacement 40–60	Hip replacement 60
Treatment group						
A	2/12	3/10	0/22	9/25	1/3	5/8
B	1/22	1/10	2/23	3/24	0/1	2/7
C	3/11	0/8	0/25	7/25	1/3	4/8
D	1/10	2/10	2/24	4/23	1/1	3/6
Total	7/45	6/38	4/94	23/97	3/10	14/29

Table III *Incidence of DVT with regimens A and B after omission of laparotomy in age group 40–60*
Treatment groups as in Table I

DVT	A	B
Present	20	7
%	34	13
Absent	38	49
Total	58	56

A vs B $\chi^2=6.45$ $p<0.02$ χ^2 test with Yates correction for continuity

week none showed signs of thromboembolic disease (post mortems were carried out in 4) Two patients changed wards one of them died in pulmonary insufficiency after pneumonectomy and no embolism was found in the post mortem Two patients could not be scanned because of plaster casts Two patients left the trial because of massive bleeding on the first p o day (included under bleeding complications) Two patients had peroperative complications that prevented further investigation Three patients were not studied because of administrative or technical failures or lack of cooperation

125I fibrinogen DVT

A significant decrease in DVT over control regimen A was found with regimen B but not with regimens C and D containing s c heparin (Tables I and II) The frequency of DVT in the latter two groups was higher than reported in the literature The probable reason for this will be discussed below The frequency of DVT in the control group (regimen A) was 25% in the same range as generally reported Almost no DVT was observed in patients 40–60 years of age undergoing laparotomy The difference between p o coumarins (A) and dextran 40 + p o coumarins (B) is more pronounced if these data are omitted (Table III)

Subcutaneous heparin

A preliminary analysis was carried out halfway through the trial and it showed that treatments C and D offered no benefit over treatment A This result differs from the literature which almost invariably shows a reduction to an incidence of 6–10% The reason for this lack of effect was therefore investigated and the ampoules prepared by the hospital pharmacy were found to contain too little heparin or placebo solution In general about 0 16 ml instead of the prescribed 0 20 ml was injected When questioned nearly all staff nurses admitted to noticing this discrepancy but being unaware of its significance they had not warned the investigators Ampoules with 0 30 ml were subsequently prepared The incidence before and after the change is shown in Table IV

The incidence of DVT in the combined gorups with s c heparin (groups C and D) had been 17 2% The incidence with 5 000 IU heparin twice daily was 5 9% The difference between before and after was not significant at the 5% level ($p=0.09$) because external factors forced us to terminate the study The frequency of DVT at a dose of 5 000 IU heparin twice daily was lower than in the acenocoumarin control group (5 9% vs 20% $0.02<p<0.05$) Groups C and D were combined for this comparison this was acceptable because their results were similar The percentage in the s c heparin group was lower than in the dextran 40 group but the difference was not significant (5 9% vs 10 4% n s)

Clinical DVT

Only 14 of 57 patients with positive scans had clinical symptoms No patient with a negative scan had clinical symptoms Ankle or calf oedema or a combination predominated Tenderness of the calf (1/14) Homan s sign (0/14) and fever (1/14) were rare or not observed

Lung scans

Lung perfusion scans were performed in all patients with positive leg scans (Table V) Six patients with

Table IV *Frequency of DVT before and after increase of heparin dosage*

DVT	Before	After
Present	16	3
%	17 2	5 9
Absent	77	50
Total	93[a]	53[b]

$\chi^2=3.27$ $p=0.09$ χ^2 test with Yates correction for continuity

[a] The patients in treatment groups C and D who had been treated with 4 000 IU twice daily were combined Patients with hip replacement were excluded because no such patients were operated on after the change to 5 000 IU twice daily

[b] Patients from treatment groups C and B were also combined after the change to 5 000 IU twice daily

3 Flanc C Kakkar V V & Clarke M B The detection of venous thrombosis of the legs using [125]I labelled fibrinogen Br J Surg 55 742 1968
4 Gallus A Hirsch J Tuttle R Trebilcock R OBrien S Carroll J Minden J & Hudecki S Small subcutaneous doses of heparin in prevention of venous thrombosis N Engl J Med 288 545 1973
5 Gruber U Dextran and the prevention of postoperative thromboembolic complications Surg Clin North Am 55 696 1975
6 Jansen H Postoperative thromboembolism and its prevention with 500 ml dextran given during operation Acta Chir Scand (Suppl) 427 1 1972
7 Kakkar V V Corrigan T & Rossard D Prevention of fatal postoperative pulmonary embolism by low doses of heparin Lancet 2 45 1975
8 Kakkar V V Corrigan T & Spinder J Efficacy of low doses of heparin in prevention of deep vein thrombosis after major surgery Lancet 2 101 1972
9 Kakkar V V Field E & Nicolaides A Low doses of heparin in prevention of deep vein thrombosis Lancet 2 669 1971
10 Kline A Hughes L Campbell H Williams A Zlosnick J & Leach K Dextran 70 in prophylaxis of thromboembolic disease after surgery a clinical oriented randomized double trial Br Med J II 109 1975
11 Kluge T H Ro J S Fretheim B & Stormorken H Thrombosis prophylaxis with dextran and warfarin in vascular operations Surg Gynecol Obstet 135 941 1972
12 Koekenberg L Experimental use of macrodex as a prophylaxis against postoperative thrombo-embolism Bull Soc Int Chir 21 501 1962
13 Van der Linde D Het voorkomen van postoperatieve diepe veneuze thrombose Thesis Utrecht 1975
14 McCarthy T G McQueen J Johnstone F D Weston J & Campbell S A comparison of low-dose subcutaneous heparin and intravenous dextran 70 in the prophylaxis of deep venous thrombosis after gynaecological surgery J Obstet Gynaecol 81 486 1974
15 A Multi unit Controlled Trial Heparin versus dextran in the prevention of deep-vein thrombosis Lancet 2 118 1974
16 Myrvold H Persson J Svensson B Wallensten S & Vikterloef K Prevention of thrombo-embolism with dextran 70 and heparin in patients with femoral neck fractures Acta Chir Scand 139 609 1973
17 Negus D Pinto D Le Quesne L Brown N & Chapman M [125]I labelled fibrinogen in the diagnosis of deep vein thrombosis and its correlation with phlebography Br J Surg 55 835 1968
18 Nicolaides A Dupont P A & Desai S Small doses of subcutaneous sodium heparin in preventing deep venous thrombosis after major surgery Lancet 3 890 1972
19 Pinto D J Controlled trial of an anticoagulant (warfarin sodium) in the prevention of venous thrombosis following hip surgery Br J Surg 57 349 1970
20 Rem J Duckert F Friedrich R & Gruber U Subcutane kleine Heparindosen zur Thromboseprofylaxe in der allgemeinen Chirurgie und Urologie Schweiz Med Wochenschr 105 827 1975
21 Sevitt S & Gallagher N J Prevention of venous thrombosis and pulmonary embolism in injured patients a trial of anticoagulant prophylaxis with phenindione in middle aged and elderly patients with fractured necks of femur Lancet 2 981 1959

Acta Med Scand 202 373-377 1977

The Effects of Four Months' Treatment with Spironolactone on Systemic Blood Pressure, Cardiac Output and Plasma Renin Activity in Hypertensive Patients

Sture Bevegård Jan Castenfors and Mats Danielson

From Medical Department IV and the Department of Clinical Physiology Sodersjukhuset Stockholm Sweden

ABSTRACT The effect of spironolactone on BP, cardiac output, plasma renin activity and urinary excretion of electrolytes has been studied in 12 hypertensive patients After 1 month of spironolactone therapy there was a significant decrease in arterial BP Urinary sodium excretion was significantly decreased and plasma renin activity increased After four months of spironolactone therapy there was no further decrease in arterial BP Cardiac output, heart rate and stroke volume were unchanged in the supine position but the calculated total peripheral vascular resistance (TPVR) was reduced indicating that the lower BP was mainly a result of dilatation of the resistance vessels During exercise there was still a significant decrease in arterial BP but this was related to a decrease in both cardiac output and TPVR

Spironolactone exerts a saluretic effect by inhibiting the action of aldosterone on distal tubuli (5 17 19 20 21) This mechanism has been used in the treatment of both hyperaldosteronism and essential hypertension (3 9 11 25 26 30 31) The BP lowering effect of spironolactone is comparable with that of other antihypertensive drugs (1 7 8 18 24)

Spironolactone has been used for many years in the treatment of hypertension but surprisingly no data have been reported on its hemodynamic effects The aim of the present investigation was to study the long term effect of spironolactone on BP cardiac output electrolytes and plasma renin activity (PRA)

STUDY BASE AND METHODS

Twelve patients (9 men and 3 women) mean age 46 years (range 33-56) were studied 11 were classified as having essential hypertension and 1 renovascular hypertension According to the WHO classification 4 patients belong to group I and 8 to group II All patients had been without antihypertensive therapy for at least 4 weeks prior to the administration of spironolactone

The patients were recruited from the Hypertension Clinic and all volunteered to take part in the investigation There were no drop-outs or complications to the examinations The patients were on a free diet but were instructed to avoid large variations in their water and food intake before the investigation Clinical evaluations were performed before and after 1 and 2 months respectively and hemodynamic evaluations after 4 months treatment with spironolactone in a dose of 50 mg×2

The significance of the difference between mean values before and after treatment has been evaluated by Student s *t* test The patient with renovascular hypertension was not included in the statistical comparison of the hemodynamic results

Clinical evaluation BP and heart rate were determined by routine clinical methods at rest supine and after 2 min in upright position Body weight was measured and blood samples were collected for determination of sodium potassium creatinine glucose uric acid and PRA Urinary sodium and potassium excretion was determined in 24-hour urine collected prior to the investigation

Hemodynamic evaluation Intraarterial BP (brachial artery) cardiac output (dye dilution technique) and heart rate (continuous ECG registration) were determined at rest supine and during standardized leg exercise on a bicycle ergometer in sitting position The work load for men was 100 W and for women 65 W PRA was determined at rest after about 1 hour in supine position after 20 min in a 45 head-up tilted position and at the end of the standardized exercise Double determinations of the cardiac output were performed both at rest supine and

Table I *Blood pressure and heart rate at rest in supine and upright position before (I) and after one month of spironolactone therapy (II) (x±S D)*

	I	II	I–II
Systolic BP (mmHg)			
Supine	180±13	155±15	−25 $p<0\ 001$
Upright	176±14	148±19	−28 $p<0\ 001$
Diastolic BP (mmHg)			
Supine	110± 9	100± 9	−10 $p<0\ 001$
Upright	123± 8	109± 8	−14 $p<0\ 001$
Heart rate (beats/min)			
Supine	82± 8	83±14	+ 1 n s
Upright	94±12	102±16	+ 8 n s

during exercise Total Hb and blood volume by the alveolar CO method were determined on the day before and on the day of the hemodynamic investigation Details on the methods for the hemodynamic investigation and blood volume determination are given elsewhere (2)

PRA was determined by a radioimmunologic technique (NEN commercial kit) based on a method published by Haber et al (10) The normal value in our laboratory for PRA is 0 97±0 70 ng ml^{-1} h^{-1} and the error of the method 0 1 ng ml^{-1} h^{-1}

RESULTS

Clinical evaluation

After 1 month of spironolactone therapy systolic and diastolic BP were significantly reduced at rest supine Heart rate showed no significant change (Table I) On transition from supine to upright position systolic BP decreased on an average 4 mmHg before and 7 mmHg after treatment the difference being non significant Diastolic BP increased 13 and 9 mmHg respectively a significant difference ($p<0\ 05$)

The increase in heart rate on transition from supine to upright position also changed significantly ($p<0\ 01$) being more marked after therapy (+19 beats/min) than before (+12 beats/min) After 2 months of spironolactone therapy the clinical evaluation demonstrated similar results

Serum potassium increased and serum sodium decreased significantly Serum creatinine glucose and uric acid showed no significant change (Table II) Urinary sodium excretion decreased significantly ($p<0\ 05$) potassium excretion was not significantly changed Body weight averaged 77 kg before and after treatment

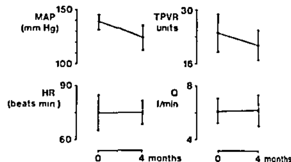


Fig 1 Hemodynamic effects of 4 months spironolactone therapy (mean±1 S D) $^{*}p<0\ 05$ $^{***}p<0\ 001$

Hemodynamic evaluation (Fig 1)

After 4 months of spironolactone therapy both systolic and diastolic intraarterial BPs were significantly reduced at rest supine (Table III) Cardiac output was essentially unchanged and consequently the calculated total peripheral vascular resistance (TPVR) was lower There was no significant change in heart rate and stroke volume

During standardized leg exercise intraarterial BP was again significantly decreased due to a lower cardiac output and TPVR but both changes were non significant (Table III)

Blood volume and plasma renin activity

Blood volume was unchanged after 4 months of spironolactone therapy (5 9±1 4 l after and 5 9±1 2 l before therapy) PRA increased significantly ($p<0\ 01$) at rest supine from 1 3±0 8 to 2 2±1 0 ng ml^{-1} h^{-1} after 1 month Mean PRA increased to 2 7±1 4 ng ml^{-1} h^{-1} after 4 months of spironolactone treatment but this increase was not significant compared with the mean values after 1 months

Table II *Serum glucose electrolyte concentration and urinary excretion of sodium and potassium before (I) and after one month of spironolactone therapy (II) (mean±1 S D)*

	I	II	I–II
S creatinine (µmol/l)	80±19	80±22	0 n s
S Na (mmol/l)	142±4	140±3	− 2 $p<0\ 05$
S K (mmol/l)	4 1±0 4	4 5±0 4	+ 0 4 $p<0\ 05$
S Cl (mmol/l)	103±2	101±2	− 2 n s
S T_{CO_2} (mmol/l)	24 8±1 4	25 1±1 7	+ 0 3 n s
S glucose (mmol/l)	4 9±0 9	4 9±1 0	0 n s
S uric acid (µmol/l)	351±54	346±52	− 5 n s
U Na (mmol/24 h)	135±37	117±35	−18 $p<0\ 05$
U K (mmol/24 h)	50±22	43±14	− 7 n s

treatment Also in head up tilted position and during exercise PRA increased significantly after 4 months (Table IV)

DISCUSSION

The BP lowering effect of spironolactone in this study is comparable with the effect of thiazide diuretics and other antihypertensive agents The reduction in BP was already maximal after 1 month of spironolactone therapy no further decrease being found at the hemodynamic evaluation after 4 months of therapy Cardiac output stroke volume and heart rate at rest supine did not differ significantly from the pretreatment values indicating that the lower BP mainly reflected a decreased TPVR During exercise however the reduction in BP was related to decreases in both cardiac output and TPVR No data have been reported in the literature concerning the hemodynamic effect of spironolactone in essential hypertension In 30 patients with Conn s syndrome Safar et al (22) showed that 3 months of spironolactone therapy induced a reduction in the cardiac output and/or TPVR both changes being non significant for the group As Conn s disease was excluded from the present study the patient groups are difficult to compare

Body weight total Hb and blood volume were unchanged after 4 months of spironolactone therapy indicating that the BP lowering effect at that time was not related to a reduction in blood volume Hunyor et al (14) on the other hand showed a significant reduction in plasma volume after 1 month of spironolactone treatment but they were not able to demonstrate any correlation between the changes in plasma volume and BP Our study does not exclude the possibility that the initial reduction in BP may be related in part to a reduction in intravascular or extracellular volumes At the clinical evaluation after 1 month of spironolactone therapy a transition from supine to upright position induced a significantly more marked increase in heart rate compared with pretreatment conditions which may be an indication of a decreased plasma volume

The BP lowering effect of spironolactone may thus be related *initially* to a reduction in cardiac output due to decreased intravascular or extracellular fluid volume (3 8) and *later* when the fluid volume and cardiac output have returned to pretreatment levels to adaptive structural vascular mechanisms (23 28) with decreased TPVR and/or lowered vascular sensitivity to vasoconstrictive stimuli (29)

The hemodynamic findings differ somewhat from those in a similar investigation of the long term effect of the sulfonamide mefruside (2) After 4 months of mefruside therapy (25 mg/day) there were indications of decreases in blood volume and stroke volume the decrease in cardiac output being not significant due to a significant increase in heart rate The significance of these differences between the hemodynamic effects of spironolactone and mefruside is however difficult to interpret since

Table III *Some hemodynamic variables in 11 patients at rest in supine position and during standardized submaximal exercise before (I) and after 4 months of spironolactone therapy (II) (mean±1 S D)*

SAP DAP MAP=systolic diastolic mean intraarterial pressure $\dot{Q}$=cardiac output HR=heart rate SV=stroke volume TPVR=total peripheral vascular resistance

	I	II	I–II	
At rest				
SAP (mmHg)	186±18	166±20	20	$p<0\ 001$
DAP (mmHg)	107±7	97±8	−10	$p<0\ 001$
MAP (mmHg)	139±7	124±12	−15	$p<0\ 001$
$\dot{Q}$ (l/min)	6 1±1 1	6 2±1 4	+ 0 1	n s
HR (beats/min)	75±10	75±7	0	n s
SV (ml)	84±13	83±15	− 1	n s
TPVR (U)	24±5	21±4	− 3	$p<0\ 05$
During exercise				
SAP (mmHg)	224±28	212±32	−12	$p<0\ 01$
DAP (mmHg)	113±13	106±12	− 7	$p<0\ 001$
MAP (mmHg)	156±19	143±18	−13	$p<0\ 001$
$\dot{Q}$ (l/min)	13 0±2 2	12 5±2 1	− 0 5	n s
HR (beats/min)	140±20	140±22	0	n s
SV (ml)	94±19	92±23	− 2	n s
TPVR (U)	13±3	12±2	− 1	n s

Table IV *Plasma renin activity (ng ml⁻¹ h⁻¹) at rest and during work before and after treatment with spironolactone (mean±1 S D)*

	Before treatment	After spironolactone 1 mo	After spironolactone 4 mo
Rest supine	1 3±0 8	2 2±1 0	2 7±1 4
Rest head up tilted 45°	2 0±1.5	–	4 0±2.2
Work sitting	3 0±2 4	–	6 3±3 5

although both groups of patients had mild to moderate essential hypertension there were minor differences in hypertensive stage sex distribution BSA and blood volume which could account for the discordant findings

The decreases in plasma sodium and urinary sodium excretion after 1 month of spironolactone therapy are in agreement with the well known saluretic effect of spironolactone (3 12 16 30) These changes and the increase in PRA may suggest a negative sodium balance but the importance of this for the BP lowering effect of spironolactone is not clear since neither sodium chloride nor plasma infusions increased the BP after such antihypertensive therapy (13) Mefruside therapy induced opposite effects on plasma sodium and potassium and also on urinary potassium excretion (2) These hemodynamic and biochemical differences may indicate that a combination of spironolactone and a diuretic drug of the sulfonamide type may have an additive effect in the treatment of hypertension Such an additive effect has been demonstrated in some studies (11 15 16 30) but is questioned in others (7 9 31)

The increase in PRA after 4 months of spironolactone therapy was similar to the increase after 4 months of mefruside therapy in the above mentioned study (2) The increase after treatment with mefruside correlated significantly with the increase in heart rate suggesting that the increase in PRA was partly related to increased sympathetic activity After spironolactone therapy however heart rate was unchanged which implies that the increase in PRA is related to intrarenal (baroreceptors or macula densa) mechanisms

The decrease in BP after 4 months of spironolactone therapy was not related to the initial PRA level ($r = -0.38$) which is in agreement with the findings of Hunyor et al (14) and Solheim et al (24) but at variance with the findings of Crane and Harris (6), Carey et al (4) Sundfjord et al (27) and Karlberg et al (18) These discrepancies may however, reflect differences in the standardization of the basal PRA value and may also be due to differences in the methods for determining PRA

There were no significant changes in plasma levels of creatinine glucose or uric acid in our patients though changes in these parameters have been reported by others (6 7 16) Side effects such as increased fatigue gynecomastia and menstrual irregularities (25) were not observed

In conclusion this study shows that spironolactone induced a reduction in BP after 4 months mainly related to a decrease in TPVR at rest

REFERENCES

1 Adlin E V Marks A D & Channik B J Spironolactone and hydrochlorothiazide in essential hypertension Arch Intern Med 130 855 1972
2 Bevegård S Castenfors J & Danielson M Haemodynamic effects of four month s mefruside therapy in hypertensive patients Acta Med Scand 201 93 1977
3 Brown J J Davies D L Ferriss J B Fraser R Haywood E Lever A F & Robertson J I S Comparison of surgery and prolonged spironolactone therapy in patients with hypertension aldosterone excess and low plasma renin Br Med J 2 729 1972
4 Carey R M Douglas J G Schweikert J R & Liddle G W The syndrome of essential hypertension and suppressed plasma renin activity Normalization of blood pressure with spironolactone Arch Intern Med 130 849 1972
5 Crabbé J Inhibition by spironolactone of the effect of aldosterone on transepithelial sodium transport In Extrarenal activity of aldosterone and its antagonists (ed W Brendel et al) p 7 Excerpta Medica Amsterdam 1972
6 Crane M G & Harris J J Effect of spironolactone in hypertensive patients Am J Med Sci 260 311 1970
7 Cranston W I The effects of spironolactone and chlorothalidone on arterial pressure Lancet 1 1161 1962
8 Douglas J G Hollifield J W & Liddle G W Treatment of low renin essential hypertension Comparison of spironolactone and hydrochlorothiazide triamterene combination JAMA 227 518 1974
9 Fagerberg S E & Goransson K En jamförande studie mellan spironolakton och klortalidon vid behandling av hypertoni Lakartidningen 71 175 1974
10 *Haber E Koerner T Page* L B Kliman B & Purnode A Application of a radioimmunoassay for angiotensin I to the physiologic measurements of plasma renin activity in normal human subjects J Clin Endocrinol 29 1349 1969
11 Hollander W Aldosterone antagonists in arterial hypertension Heart Bull 12 108 1963
12 Hollander W Chobanian A V & Williams R W Potential effect of the aldosterone antagonists in hypertension In Clinical use of aldosteron antagonists (ed F C Bartter) p 169 Thomas Springfield Ill 1969
13 Hollander W & Wilkins R W The pharmacology and clinical use of rauwolfia hydralazine thiazides and aldosterone antagonists in arterial hypertension Prog Cardiovasc Dis 8 291 1966
14 Hunyor S N Zweifler A J Hansson L Schork A & Ellis C Effect of high dose spironolactone and chlorthalidone in essential hypertension Relation to

plasma renin activity and plasma volume Aust NZ J Med 5 17 1975
15 Ibsen H The effect of potassium chloride and spironolactone on thiazide induced potassium depletion in patients with essential hypertension Acta Med Scand 196 21 1974
16 Jamil M A A comparative study of spironolactone and hydroflumethiazide alone and together in the treatment of hypertension Guys Hosp Rep 120 207 1971
17 Kagawa C M Bouska D J Anderson M L & Krol W F Pharmacological properties of a mineralocorticoid antagonist (SC 14266) Arch Int Pharmacodyn Ther 149 8 1964
18 Karlberg B E Kågedal B Tegler L & Tolagen K Renin concentrations and effects of propranolol and spironolactone in patients with hypertension Br Med J 1 251 1976
19 Liddle G W Aldosterone antagonists A M A Arch of Internal Medicine 102 998 1958
20 — Specific and non specific inhibition of mineral corticoid activity Metabolism 10 1021 1961
21 Ross E Aldosterone and its antagonists Clin Pharmacol Ther 6 65 1965
22 Safar M Tomowa J Menard J & Millez P Hemodynamique du syndrome de Conn Etude après spironolactone et apres traiment chirurgical In Extrarenal activity of aldosterone and its antagonists (ed W Brendel et al) p 77 Excerpta Medica Amsterdam 1972
23 Sivertsson R The hemodynamic importance of structural vascular changes in essential hypertension Acta Physiol Scand (Suppl) 343 1973
24 Solheim S B Sundsfjord J A & Giezendanner L The effects of spironolactone (Aldactone®) and methyldopa in low and normal renin hypertension Acta Med Scand 197 451 1975
25 Spark R F Melby J C Aldosteronism in hypertension The spirnolactone response test Ann Intern Med 69 685 1968
26 Storm Mathisen H Antihypertensiv terapi Tidsskr Nor Laegeforen 92 1331 1972
27 Sundsfjord J A Jørgensen H & Aakvaag A Prognostic value of renal vein renin activity in the treatment of hypertensive patients with aldactone In Extrarenal activity of aldosterone and its antagonists (ed W Brendel et al) p 89 Excerpta Medica Amsterdam 1972
28 Weiss L Aspects of the relation between functional and structural cardiovascular factors in primary hypertension Acta Physiol Scand (Suppl) 409 1974
29 Wessels F & Zumkley H Untersuchungen uber den Einfluss des intra und extrazellularen Elektrolytstoffwechsels auf die Gefässreagibilitat Z Kreislaufforsch 59 427 1969
30 Winer B M Lubbe W F & Colton Th Antihypertensive actions of diuretics JAMA 204 117 1968
31 Wolf R L Mendlowitz M Roboz J Styan G P H Kornfeld P & Weigl A Treatment of hypertension with spironolactone JAMA 198 1143 1966

Acta Med Scand 202 379–384 1977

Effect of Saluretic Therapy on Muscle Content of Water and Electrolytes in Relation to Hemodynamic Variables

Sture Bevegård Jan Castenfors Mats Danielson and Jonas Bergstrom

From the Department of Clinical Physiology and Medical Department IV Sodersjukhuset and Rheumatological Research Laboratory St Erik s Hospital Stockholm Sweden

ABSTRACT Muscle content of water and electrolytes (needle biopsy), intraarterial BP and cardiac output (dye dilution technique) were measured in 12 patients with essential hypertension before and after 4 months of mefruside therapy (25 mg/day) Before therapy there were no significant differences in muscle tissue electrolyte and water content compared with normotensive subjects No correlation was found between central hemodynamic variables and the electrolyte and water content of muscle tissue either before or after therapy After 4 months of mefruside therapy muscle tissue water showed a mean decrease which was not significant Serum potassium and muscle potassium content decreased significantly but there was no significant change in intracellular potassium concentration Intracellular sodium concentration increased significantly while muscle sodium content showed a mean increase which was not statistically significant The change in intracellular sodium concentration showed a significant negative correlation with the decrease in mean arterial BP The change in total cellular water content showed a significant negative correlation to the changes in total peripheral vascular resistance Saluretic therapy seems to induce counterregulatory mechanisms that interfere with the hypotensive effect

The connection between sodium and hypertension is well known In 1920 Allen (1) showed that increased BP could be decreased if sodium intake was reduced and not chloride as proposed earlier Similar results were obtained in animals (17) and humans (20) It has also been observed that increased salt intake may cause hypertension both in animals and in humans (22) A genetic factor seems to play an important role in the development of salt hypertension (12) In the spontaneously hypertensive rat a correlation was observed between salt intake and systolic BP (21)

Increased salt content in single cells and tissue especially in the arterial wall has been demonstrated in animal experiments in which hypertension was induced (13 23 25) An increase in the content of both water sodium and potassium in the arterial walls of hypertensive animals has also been reported (18 24) but Feigl et al (15) found an increase in the water content only

In hypertensive man an increased sodium content has been found in red blood cells (27) and an increased sodium and water content in muscle tissue (22) Saluretic therapy induced a decrease in muscle water and potassium but less consistent changes in muscle sodium both in hypertensive and in normal subjects (3 6 7 25) In normotensive dogs on the other hand no changes were found in sodium or water content of arterial or venous wall after 6–8 weeks of saluretic treatment (28)

The increased BP in salt hypertension is mainly attributed to an increase in calculated total peripheral vascular resistance (TPVR) secondary to salt and pressure induced changes in the arterial wall (19) but increased sensitivity to sympathetic stimuli has also been proposed (9 27)

No studies seem to have been published on the relationship between hemodynamic variables and the salt and water content of muscle tissue in patients with essential hypertension The purpose of this study is to determine whether a relationship exists between TPVR and the salt and water content of muscle tissue and if so to investigate the effect of saluretic therapy on this relationship

Table I *Anthropometric and clinical data on 12 untreated patients with essential hypertension*

Pat no	Age (y)	Sex	Height (m)	Weight (kg)	Hyper tensive stage	Blood volume (l) Estimated	Blood volume (l) Predicted	S creatinine (mg/100 ml)	Mean arterial BP (rest supine) (mmHg)
1	55	♀	1 58	55	I	4 0	3 8	1 1	145
2	57	♀	1 61	51	I	3 5	3 5	0 8	133
3	40	♂	1 82	79	I	6 1	6 4	1 3	145
4	49	♀	1 66	75	I	5 1	5 2	0 7	121
5	41	♂	1 76	69	II	4 7	5 6	1 0	138
6	54	♂	1 77	73	II	4 7	5 7	1 3	154
7	51	♀	1 57	49	I	4 0	3 4	1 0	140
8	42	♂	1 79	88	II	5 8	6 9	0 9	137
9	44	♂	1 76	80	II	4 3	6 3	1 1	138
10	56	♂	1 80	75	I	5 8	5 9	0 9	133
11	43	♂	1 70	55	I	5 3	4 3	1 0	140
12	39	♀	1 54	66	II	4 4	4 6	0 7	118
Mean	48		1 70	68		4 9	5 1	1 0	137
1 S D	±7		±10	±13		±0 8	±1 2	±0 2	±11

* Criteria proposed by the WHO (1962)

PATIENTS AND METHODS

Twelve patients with essential hypertension 7 men and 5 women were included in the study (mean age 48 years range 39–57) Seven patients belonged to group I according to WHO s classification and five to group II (Table I) Blood volume and kidney function were normal

Before treatment intraarterial BP and cardiac output (dye dilution technique) were measured at rest in supine position after about 6–10 min in sitting position and finally during standardized leg exercise on a bicycle ergometer The hemodynamic studies and determination of serum were performed in the morning the patients at 8 a m and the study being completed by about a m Urinary excretion of sodium and potassium was measured during the preceding 24 hours Double determination of blood volume by the alveolar CO method was performed within 3 days of the hemodynamic investigation Needle biopsy of muscle tissue was performed on the day after the hemodynamic investigation The measurements were repeated after 4 months of mefruside therapy (25 mg/day) Details of the methods for hemodynamic investigation are described elsewhere (8)

The muscle biopsies from m quadriceps femoris were performed at 8–10 a m The needle biopsy specimens were taken alternately from both legs before and after the period of diuretic administration On each occasion sampling was done at two sites 14–16 cm and 20–22 cm proximally to patella

Each specimen which weighed 20–80 mg was divided into 2–4 pieces weighing 10–20 mg Visible fat and connective tissue were rapidly removed by dissection and the specimens were weighed on an electromagnetic balance The specimens were dried at 90°C and weighed again Neutral fat was extracted with petroleum ether and the pieces were weighed once more The water and fat contents were calculated The details of the biopsy technique weighing procedure and fat extraction have been described earlier (2)

Sodium potassium and magnesium contents were determined by atomic absorption spectrophotometry and chloride was measured by electrometric titration (7) Serum electrolytes and protein were analysed according to routine methods (Auto Chemist) separate samples of heparinized plasma were used for potassium and magnesium determination by emission and absorption

Table II *Effect of four months mefruside treatment on central hemodynamics at rest supine and during exercise sitting (mean±1 S D)*

	Rest Without therapy	Rest Mefruside	Rest Difference	Rest p	Exercise Without therapy	Exercise Mefruside	Exercise Difference	Exercise p
Mean arterial pressure (mmHg)	137±10	124±12	−13	<0 001	160±24	147±19	−13	<0 025
Cardiac output (l/min)	5 2±1 0	4 8±0 7	−0 4	<0 1	11 7±1 7	11 0±1 9	−0 7	<0 1
Total peripheral vascular resistance (U)	26 9±5 0	26 6±5 0	−0 3	<0 1	14 0±3 8	13 9±3 8	−0 1	<0 1

Table III *Effect of four months mefruside treatment on serum electrolytes and urinary electrolyte excretion (mean±1 S D)*

	Without therapy	Mefruside	Differ ence	*p*
S Na (mEq/l)	138 1±2 1	139 2±2 0	+1 1	n s
S K (mEq/l)	4 0±0 3	3 6±0 4	−0 4	<0 05
S-Cl (mEq/l)	104 2±2 2	103 2±1 5	−1 0	n s
S standard bicarbonate (mEq/l)	23 5±1 2	24 8±1 3	+1 3	<0 025
Urine volume (ml/24 h)	1 258±587	1 025±424	−233	<0 025
U Na (mEq/24 h)	139±65	141±43	+2	n s
U K (mEq/24 h)	49±20	62±20	+13	<0 05

flame spectrophotometry respectively Standard bicarbonate pH and P_{CO_2} were determined in whole blood by blood gas analyser (IL 213) Tissue water and electrolyte contents were referred to 100 g fat free solids (FFS) The determination of extra and intracellular water was based on the chloride method Chloride is freely diffusible across the skeletal muscle fibre membrane and is distributed according to Nernst s equation (11) Taking the resting membrane potential of muscle in normal man to be 87 2 mV (10) the Cl_e/Cl_i ratio calculated from Nernst s equation will be 26/1 if the total water and chloride content of the muscle tissue and the extracellular concentration (obtained by correcting the plasma chloride concentration for a Donnan factor and a factor for plasma water (2)) are known extra and intracellular water volumes and intracellular electrolyte concentrations can be calculated (5 16)

RESULTS

Central hemodynamics (Table II)

Mean arterial BP decreased significantly at rest in supine position in sitting position and during standardized leg exercise Cardiac output at rest showed a minor but non significant decrease from 5 2 to 4 8 l/min neither did it change significantly in sitting position at rest or during exercise The TPVR at rest showed a slight non significant decrease (from 26 9 to 26 6 U) here too there was no significant change in sitting position at rest or during exercise

Plasma electrolytes and urinary electrolyte excretion (Table III)

Plasma There were no significant changes in plasma sodium and chloride concentrations Standard bicarbonate increased and plasma potassium concentration decreased significantly

Urinary excretion Potassium excretion in urine increased significantly sodium excretion did not change significantly and urine volume decreased significantly

Electrolytes and water in muscle tissue (Table IV)

Compared with normotensive normal subjects (6 men and 4 women aged 24–35 examined in the

Table IV *Muscle tissue water and electrolyte content in 10 normotensive and 12 hypertensive subjects before and after mefruside therapy (mean±1 S D)*
FFS=fat free solids

	Normotensive subjects*	Hypertensive patients			
		Without therapy	Mefruside therapy	Difference	*p*
Total water (ml/100 g FFS)	336±12	347±11	344±14	−3	n s
Extra cellular water (ml/100 g FFS)	40±10	45±8	40±9	−5	n s
Intracellular water (ml/100 g FFS)	297±9	302±12	303±6	+1	n s
Chloride (mEq/100 g FFS)	6 0±1 2	6 5±0 9	6 0±1 0	−0 5	n s
Sodium (mEq/100 g FFS)	9 3±1 5	9 6±1 3	9 8±1 2	+0 2	n s
Potassium (mEq/100 g FFS)	46 7±0 9	47 3±1 8	46 6±1 5	−0 7	<0 025
Magnesium (mEq/100 g FFS)	8 9±0 2	8 9±0 3	8 8±0 2	−0 1	n s
Sodium (mEq/l intracellular H_2O)	12 6±2 0	11 2±1 5	13 7±3 0	+2 5	<0 005
Potassium (mEq/l intracellular H_2O)	157±5	157±3	154±5	−3	n s

* From Bergstrom (4)

Table V *Correlation between supine mean arterial BP (MAP mmHg) total peripheral vascular resistance index (TPRI U) and muscle tissue water and electrolyte content before and after mefruside therapy (r values)*
FFS=fat free solids

Independent parameter	Dependent parameter			
	Without therapy		Mefruside	
	MAP	TPRI	MAP	TPRI
Total muscle water (ml/100 g FFS)	+0 20	−0 12	−0 14	−0 16
Intracellular water (ml/100 g FFS)	+0 40	−0 24	−0 12	0 00
Sodium (mEq/100 g FFS)	−0 24	−0 18	+0 25	−0 07
Sodium (mEq/l intracellular H_2O)	−0 29	−0 21	+0 04	+0 07

same way by Bergstrom (4)) there was no significant difference in tissue electrolytes and water content before therapy

After four months of mefruside therapy there was no significant change in sodium content expressed as mEq/100 g FFS But sodium content/l intracellular water increased significantly The total potassium content/100 g FFS was significantly decreased but potassium content/l intracellular water showed only a slight non significant fall No significant change was found in either chloride or magnesium

Correlation between mean arterial BP TPVR and tissue sodium and water before and after therapy (Table V)

There were no significant correlations between tissue sodium or water and mean arterial pressure and

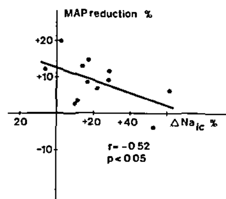


Fig 1 Correlation between changes in MAP and changes in intracellular sodium after four months of treatment with mefruside

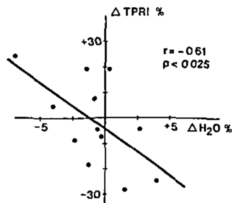


Fig 2 Correlation between changes in total peripheral vascular resistance index (*TPRI*) and changes in muscle tissue water after four months treatment with mefruside

no significant correlation to total peripheral vascular resistance either before or after four months of mefruside therapy

Correlation between the changes in arterial BP and TPVR and the changes in tissue water and sodium content induced by four months of mefruside therapy (Table VI)

A significant negative correlation was found between the reduction of supine arterial BP and the *increase in intracellular sodium* (Fig 1) but there was no correlation between changes in arterial pressure and intracellular water or total tissue water The changes in TPVR showed a significant negative correlation to total water content (Fig 2) but not to the change in intracellular water or sodium content

DISCUSSION

With respect to muscle electrolyte and water content the untreated hypertensive patients in this

Table VI *Correlation between changes (%) induced by mefruside treatment in mean arterial BP (MAP) and total peripheral vascular resistance index (TPRI) and changes (%) in muscle tissue water and electrolytes (r values)*

Independent parameter	Dependent parameter	
	MAP	TPRI
Muscle tissue water	−0 06	−0 61*
Intracellular water	+0 23	−0 21
Intracellular sodium	+0 52*	+0 08

*$p<0.05$

study did not differ significantly from normotensive healthy subjects studied by similar techniques This is contrary to the findings of Villamil et al (26) that hypertensive patients have significantly increased total tissue water intracellular water muscle sodium content and intracellular sodium Increased muscle tissue water in hypertensive subjects with a mean age of 66 years has also been reported by Bergstrom et al (7) A possible explanation for the discrepancy may be the age of the hypertensive patients since tissue content of water and extracellular electrolytes (sodium and chloride) gradually increase with age (14) In the present study population the age difference between the hypertensive group (44 years) and the normotensive control group (29 years) was smaller which may explain the absence of a significant difference

After saluretic therapy with hydrochlorothiazide 100 mg/day Villamil et al (26) demonstrated a significant decrease in both muscle tissue water and intracellular water and also in sodium and potassium content in muscle tissue in hypertensive subjects This agrees with the findings of Bergstrom et al (7) who demonstrated a significant decrease in total and intracellular muscle tissue water after four months of mefruside treatment in eight hypertensive patients Four months of mefruside therapy induced similar changes in muscle sodium potassium and water content in our patients But except for muscle potassium content and intracellular sodium concentration the changes were not statistically significant

The increase in intracellular sodium content probably occurs in an exchange for potassium lost from the muscle cell This is supported by the finding that the potassium sparing drug amiloride preserves the intracellular electrolyte milieu during thiazide administration (4) The changes demonstrated in muscle potassium content are very small the decrease in the intracellular concentration being only 1 5% which is considerably less than the decrease in plasma potassium by 10% Thus the hypokalemia seems to be more dependent on the alkalosis than on the intracellular potassium depletion The hypokalemic alkalosis demonstrated after four months of mefruside therapy in these patients agrees with earlier results (6 7)

The significant mefruside induced decrease in arterial BP was related to a slight non significant mean decrease in both cardiac output and TPVR (8) The decrease in mean arterial BP showed a significant negative correlation with the increase in intracellular sodium concentration—patients with the most marked increase in intracellular sodium showed a less marked decrease in BP (Fig 1) A significant negative correlation was also found between the changes in TPVR and total tissue water—the patients with a decrease in muscle tissue water tended to show an increased peripheral vascular resistance and vice versa (Fig 2)

The importance of and the explanation for this paradoxical outcome are obscure but may have to do with counterregulatory mechanisms induced by the prolonged saluretic therapy A reduction of extra and intravascular volumes may trigger sympathetic mechanisms which could be responsible for the tendency to increased peripheral resistance and also induce secondary aldosteronism which may contribute to the increase in intracellular sodium After the four months of mefruside therapy significant increases were observed in heart rate and plasma renin activity which may indicate increased sympathetic activity and secondary aldosteronism respectively

No significant relationship was found between either BP or TPVR and muscle tissue water and electrolytes either before or after therapy These results do not contradict the relationship that has been demonstrated in several animal experiments between electrolytes and/or water in arterial wall and hypertension But the importance of these changes in arterial wall electrolyte composition and water content as a primary hypertensive mechanism is still uncertain It has been shown by Hollander et al (18) that 4–12 weeks after induced aortic coarctation in dogs sodium chloride and water content in the aortic wall were significantly increased in the upper part of the aorta with increased BP but that below the coarctation the values were normal This suggests that the changes may be secondary to—and not the cause of—the increased BP

REFERENCES

1 Allen F M Arterial hypertension JAMA 71 652 1920
2 Bergstrom J Muscle electrolytes in man Determined by neutron activation analysis on needle biopsy specimens A study on normal subjects kidney patients and patients with chronic diarrhoea Scand J Clin Lab Invest (Suppl) 68 1962
3 — Effekten av Hygroton® på cellens elektrolyter hos patienter med essentiell hypertoni Läkartidningen 61 13 1964

Table V *Correlation between supine mean arterial BP (MAP mmHg) total peripheral vascular resistance index (TPRI U) and muscle tissue water and electrolyte content before and after mefruside therapy (r values)*

FFS–fat free solids

Independent parameter	Dependent parameter			
	Without therapy		Mefruside	
	MAP	TPRI	MAP	TPRI
Total muscle water (ml/100 g FFS)	+0 20	−0 12	−0 14	−0 16
Intracellular water (ml/100 g FFS)	+0 40	−0 24	−0 12	0 00
Sodium (mEq/100 g FFS)	−0 24	−0 18	+0 25	−0 07
Sodium (mEq/l intracellular H_2O)	−0 29	−0 21	+0 04	+0 07

same way by Bergstrom (4)) there was no significant difference in tissue electrolytes and water content before therapy

After four months of mefruside therapy there was no significant change in sodium content expressed as mEq/100 g FFS But sodium content/l intracellular water increased significantly The total potassium content/100 g FFS was significantly decreased but potassium content/l intracellular water showed only a slight non significant fall No significant ... was found in either chloride or magnesium

Correlation between mean arterial BP TPVR and tissue sodium and water before and after therapy (Table V)

There were no significant correlations between tissue sodium or water and mean arterial pressure and no significant correlation to total peripheral vascular resistance either before or after four months of mefruside therapy

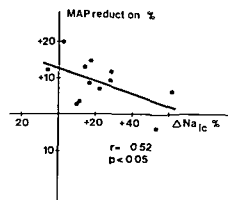


Fig 1 Correlation between changes in MAP and changes in intracellular sodium after four months of treatment with mefruside

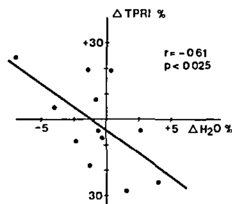


Fig 2 Correlation between changes in total peripheral vascular resistance index (*TPRI*) and changes in muscle tissue water after four months treatment with mefruside

Correlation between the changes in arterial BP and TPVR and the changes in tissue water and sodium content induced by four months of mefruside therapy (Table VI)

A significant negative correlation was found between the reduction of supine arterial BP and the increase in intracellular sodium (Fig 1) but there was no correlation between changes in arterial pressure and intracellular water or total tissue water The changes in TPVR showed a significant negative correlation to total water content (Fig 2) but not to the change in intracellular water or sodium content

DISCUSSION

With respect to muscle electrolyte and water content the untreated hypertensive patients in this

Table VI *Correlation between changes (%) induced by mefruside treatment in mean arterial BP (MAP) and total peripheral vascular resistance index (TPRI) and changes (%) in muscle tissue water and electrolytes (r values)*

Independent parameter	Dependent parameter	
	MAP	TPRI
Muscle tissue water	−0 06	−0 61*
Intracellular water	+0 23	−0 21
Intracellular sodium	+0 52*	+0 08

*$p<0.05$

Acta Med Scand 202 385–388 1977

Controlled Clinical Study on Antihypertensive Treatment with a Diuretic and Methyldopa Compared with a β-Blocking Agent and Hydralazine

Mogens Hansen Ole Paaske Hansen and Jorgen Lindholm

From Department of Internal Medicine C Bispebjerg University Hospital Copenhagen Denmark

ABSTRACT Twenty eight previously untreated patients with essential hypertension were included in a randomized double blind cross over study comparing the results of treatment with the established combination of a thiazide and methyldopa (regimen A) with the combination of a β receptor blocker and hydralazine (regimen B) Three patients each developed intolerable side effects on each regimen but they were all treated successfully on the alternative regimen The remaining 22 patients obtained a significant reduction in BP at rest and during exercise on both treatments with no significant difference between the two schedules Heart rate was significantly reduced at rest and during exercise with regimen B while a significant reduction was also obtained following exercise on regimen A There was no significant difference between the two regimens as to tolerable side effects during treatment which were registered in about 60% of the patients on each scheme However 64% of the patients were treated satisfactorily without side effects on either regimen It is concluded that the combination of a β blocking agent and hydralazine is without obvious advantages compared with the combination of thiazide and methyldopa in obtaining initial BP control in patients with essential hypertension

The beneficial effect on survival of treating arterial hypertension is now well documented (7 8) The prevalence of raised BP is not exactly known but there is no doubt that hypertension and antihypertensive therapy are major medical and economic issues

During the last decade β receptor blocking agents have been used increasingly in the routine treatment of hypertension It has not been demonstrated convincingly that such treatment is generally superior to previous therapy with diuretics and methyldopa This is also true of the combined therapy with a peripheral vasodilator and a β blocking drug which has been advocated as being especially useful (3 4 5 9) No studies comparing these two treatment combinations have been published so far

This paper presents the results of such a controlled clinical study on antihypertensive treatment with a diuretic (cyclopenthiazide Navidrex K®) and methyldopa (Dopamet®) compared with treatment with a β blocking drug (oxprenolol Trasicor®) and a peripheral vasodilator (hydralazine Apresolin®)

PATIENTS

A priori it was thought likely that the efficacy of the two regimens in reducing BP might not differ very much and that the incidence of side effects and the clinical tolerability *might well be the decisive issues Accordingly in planning* this study we took a difference in patients preference of 50% for either treatment as the smallest difference of clinical significance With a probability of 5% to demonstrate such a difference or more by random error ($p<0.05$ type 1 error) and with a probability of 0.5% of failing to demonstrate the same difference ($p<0.005$ type 2 error) we calculated that the smallest number of patients included should be 28

All patients who had not previously received antihypertensive therapy were eligible for study provided measurement of the resting supine diastolic BP on three occasions at one week intervals yielded a mean value higher than that given in Table 1 Thirty patients were included initially Two were excluded during the trial—one sustained an acute myocardial infarction and one failed to present himself for the final visit The trial was concluded when the first 28 patients had finished both treatment periods All subjects were ambulatory and

Table I *Limits for diastolic blood pressure*

Age (y)	Diastolic BP at rest (mmHg) ♂	♀
<40	95	100
40–49	100	105
50–59	105	110
60–70	110	115

treated in the Out Patient Clinic Their age and sex distribution and median BP are given in Table II

Patients with one of the following criteria were excluded 1) Evidence of secondary hypertension as established from the history and routine examination 2) Severe and rapidly progressive hypertension 3) Clinical signs of cardiac incompensation 4) A history of bronchial asthma 5) Presence of bradycardia (heart rate below 60/min) or cardiac arrhythmias 6) Obesity (body weight in excess of normal weight (Natvig s tables) + 25%) 7) Clinical or biochemical evidence of liver disease 8) Pregnancy or treatment with oral contraceptives

All patients gave informed consent to participation

METHODS

Design of the trial The study was planned as a double blind cross-over trial with open therapeutic control Patients who fulfilled the above criteria were randomized to start with either treatment A (thiazide+methyldopa) or B (oxprenolol+hydralazine) Details of the two regimens are given in Table III The dosage was stepwise increased at intervals until either the resting diastolic BP had below the levels given in Table I as measured on each of two days with two-week intervals or until the patient had been on the highest dose of the regimen for 4 weeks without satisfactory control of the BP After completion of this period (period 1) no antihypertensive treatment was given for one week After this wash-out period during which no placebo was given the patient was started on the alternate scheme (period 2)

Potassium chloride was supplemented with the thiazide tablets (600 mg/0 25 mg) Commercially available tablets not matched for colour or size were dispensed in memory packs from the Out Patient Clinic and were not marked with brand or manufacturer

Procedures BP was invariably measured on the right arm with the subject in the supine position after 10 min rest immediatel thereafter standing and after 5 min exercise on a bicycle ergometer (males 600 females 450 kpm/min) Diastolic pressure was recorded when the Korotkoff sounds disappeared BP measurements were performed using the same mercury manometer with a standard cuff by the same nurse throughout She was unaware of the current treatment and previous BP values The same physician was in charge of adjusting the dosage of antihypertensive medication on the basis of the actual supine resting BP

Laboratory analyses The following variables were monitored during the trial ECG potassium creatinine uric acid calcium phosphorus in serum fasting blood glucose X ray examination of the chest These variables were checked initially and at the end of each period

Isotope renography was performed in all subjects and if indicated additional urography and/or renal angiography to exclude renal arteriostenosis

Side effects were evaluated in two ways Firstly from the patients preference for either treatment A or B The patients were asked—after completion of the entire trial—during which of the two periods they had felt most comfortable Secondly questionnaires on side-effects noted with both relevant and dummy questions were to be completed at home before each visit

Statistics Fisher's exact test and the Wilcoxon rank sum test were used for the statistical analyses

RESULTS

Blood pressure Median BP values before treatment and after period 1 (schedule A or B) and period 2 (schedule B or A) are given in Table II Following the wash out period BPs were lower than the initial values but did not differ significantly between the two groups The values recorded at the end of period 2 were almost identical to those found after period 1 The dosage levels in treatments A and B at which satisfactory control of BP was achieved appear from Table III The reduction of BP was significant ($p<0\ 001$) in both groups and during both

Table II *Age BP (median values) and sex ratio in patients at rest (R) and during exercise (E) randomi ed to start with treatment A or B*

Three patients did not complete treatment A or B respectively and have been excluded from the respective calculations

Initial therapy	N	Age (y)	♂	♀	Blood pressure (mmHg) Before period 1 R	E	After period 1 R	E	After wash-out R	E	After period 2 R	E
A	14	52	6	8	190/118	239/130	148/85	185/100	175/103	220/110	159/95	195/105
B	14	49	7	7	202/123	225/126	155/85	185/90	170/110	210/120	153/90	180/107

Table III *Details of regimens A and B and dosage level at which BP was well controlled*

Treatment A		Treatment B		Control of BP achieved (no of pats)	
Cyclopenthiazide (mg)	Methyldopa (mg)	Oxprenolol (mg)	Hydralazine (mg)	Treatment A	Treatment B
0 5		80 b i d		6	7
0 5	250 b i d	80 t i d		13	3
0 5	250 t i d	80 t i d	25 t i d	3	9
0 5	500 b i d	80 t i d	50 t i d	1	2
0 5	500 t i d	80 t i d	75 t i d	2	4

regimens but did not differ between the two treatments ($p>0$ 05) The median reduction of BP during the two regimens is shown in Table IV

Heart rate Table IV also shows the overall reduction in heart rate during the two schemes The reduction in resting heart rate was significant following treatment with oxprenolol and hydralazine ($p<0$ 05) and the decrease in postexercise heart rate was significant after both treatments ($p<0$ 05)

Side effects In each group 3 patients did not complete scheme A or B respectively because of intolerable side effects (Table V) No patient gave a positive answer to dummy questions in the questionnaires There was no significant difference between the two treatments ($p>0$ 05) It is however of some interest that BP could be controlled satisfactorily without side effects in 64% of the patients by treatment with either A *or* B Furthermore the six patients who developed intolerable side effects could be treated satisfactorily on the alternative scheme Patients preference for the two regimens appears from Table V The difference is not significant ($p>0$ 05)

Orthostatic hypotension was not observed in any subject In no case were side effects noted from the sudden withdrawal of oxprenolol

Laboratory results The values of all the above mentioned laboratory tests were normal and did not change significantly during the course of the trial Heart size increased in one patient during treatment with oxprenolol but no such finding was made in any other subject at X ray examination of the chest

Table IV *Effect of treatment*

Median reduction in	Treatment A	Treatment B
BP (mmHg)		
At rest	44/30*	41/28
During exercise	50/23*	41/23*
Heart rate (beats/min)		
At rest	1	14*
During exercise	13	25*

* Significantly different from pretreatment values ($p<0$ 01)

DISCUSSION

The antihypertensive effects of thiazides methyldopa β blocking agents and hydralazine are well established as reported in a considerable number of papers

Hydralazine has many properties making it an ideal antihypertensive drug It acts directly on the arterioles where it has a relaxing effect on the muscle layer of the vessel wall with a resultant decrease in peripheral resistance It induces increases in heart rate and stroke volume (6) A com

Table V *Side effects and preference as reported by patients*

Figures within parentheses denote unacceptable side effects (i e so severe as to prompt discontinuation of treatment)

	Treatment A	Treatment B
Fatigue	7	5 (1)
Mental depression	3	4 (1)
Blurred vision	0	4
Dysbasia	0	2
Insomnia	1	2
Dizziness	1 (1)	4 (2)
Impotence	1	1
Skin rash	1 (1)	0
Diarrhea	2 (1)	0
Dyspnea	0	1 (1)
Total no of		
Symptoms	16 (3)	23 (5)
Patients	15 (3)	15 (3)
Patients preference*	15	8

* Five patients had no preference

bination with a β blocking drug might control these side effects on the heart besides augmenting the antihypertensive effect (6)

The present trial was designed as a double blind cross over study in order to compare the combina tion of a β blocking agent and hydralazine with the well established combination of a diuretic and methyldopa In choosing a wash out period of 1 week consideration was paid to the fact that sev eral patients might have a rather high BP Thus it was felt that sudden withdrawal of treatment fol lowed by a prolonged period without therapy was ethically unacceptable Alternatively only period 1 could be considered if the results indicated an influ ence of the treatment in period 1 on the results in period 2 Since a marked increase in BP did in fact occur during the one week wash out period without any difference between the two groups we consider the cross over to be valid

Our results show that both regimens elicited a satisfactory reduction of BP in all the patients who did not develop intolerable side effects Since the reduction in median BP did not differ significantly between the two regimens the comparison of side effects is valid The number of patients with in tolerable as well as tolerable side effects was the same during both treatments More symptoms were reported for the combination of oxprenolol and hydralazine and more patients had a preference for combination of the diuretic and methyldopa owever these differences were not statistically significant

We analysed patients preference according to age sex and dosage levels A tendency was found for young subjects and women to prefer regimen B and for elderly subjects and men to prefer regimen A Further most patients with a preference for the latter obtained satisfactory control of BP with less than 1 g methyldopa/day The design of this trial does not allow statistical analyses of these parame ters but they should be taken into account in future studies

Patients treated with oxprenolol and hydralazine achieved a significant decrease in heart rate both at rest and after exercise Heart rate during exercise was also reduced with diuretic and methyldopa This was rather unexpected as it is generally as sumed that neither diuretics nor methyldopa have any effect on heart rate Barritt et al (1) however found the same decrease in heart rate during treat ment with methyldopa

Freeman and Knight (2) have studied the effect of oxprenolol+hydralazine compared with that of methyldopa alone in 26 patients They found an identical antihypertensive effect of the two modes of therapy but a marked improvement in the pa tients mental condition occurred after transferal to treatment with oxprenolol However these patients were apparently specifically asked about this point and their answer may thus have been biased

In conclusion it may be stated that the combined treatment with a β blocking drug (oxprenolol) and a peripheral vasodilator (hydralazine) is as effective as the established treatment with a diuretic (cyclopenthiazide) and methyldopa in reducing ele vated BP without significant differences in the in cidence of side effects In fact there was a tendency in favour of thiazide+methyldopa However this study does not answer the question whether these two modes of treatment differ in the long term treatment of hypertension Such studies are clearly needed

ACKNOWLEDGEMENT

The work was supported by a grant from Kong Christian X Fond The antihypertensive drugs were supplied by Ciba-Geigy and Dumex Copenhagen

REFERENCES

1 Barritt D W Marshall A J & Heaton S Compari son of oxprenolol and methyldopa in hypertension Lancet 1 503 1976
2 Freeman J W & Knight L W Oxprenolol and hydralazine in the treatment of hypertension Med J Aust (Special Suppl) 1 12 1975
3 Gilmore E Weil J & Chidsey C Treatment of essential hypertension with a new vasodilator in combination with beta adrenergic blocade N Engl J Med 282 521 1970
4 Goble A J Diuretics beta blockers and vasodila tors—dosage in mild and moderate hypertension Med J Aust (Special Suppl) 1 14 1975
5 Hansson L Olander R Åberg H Malmcrona R & Westerlund A Treatment of hypertension with propranolol and hydralazine Acta Med Scand 190 531 1971
6 Koch Weser J Hydralazine N Engl J Med 295 320 1976
7 Veterans Administration Cooperative Study Group on Antihypertensive Agents Effects of treatment on morbidity in hypertension I JAMA 202 116 1967
8 — Effects of treatment on morbidity in hypertension II JAMA 213 1143 1970
9 Zacest R Gilmore E & Koch Weser J Treatment of essential hypertension with combined vasodilation and beta adrenergic blockade N Engl J Med 286 617 1972

Acta Med Scand 202 389–392 1977

Effects of Beta-Adrenergic Blockade on Diurnal Variability of Blood Pressure and Plasma Noradrenaline Levels

P W de Leeuw H E Falke T L Kho R Vandongen A Wester and W H Birkenhäger

From the Department of Internal Medicine Zuiderziekenhuis Rotterdam the Netherlands

ABSTRACT The effect of propranolol on diurnal variability of BP and plasma noradrenaline levels was assessed in 15 patients with uncomplicated essential hypertension While total variability and average noradrenaline level remained unaltered the pressor range (difference between basal and maximum BP readings) was reduced in most patients An inverse relationship was observed between changes in pressor range and noradrenaline

Twenty four hour blood pressure patterns and the influence of sleep in particular have been extensively studied both in normotensives and in hypertensives (2 3 4 20 22 23 26) Direct as well as indirect automatic techniques have been used

The present study was designed to assess the influence of the β blocker propranolol on this aspect of BP control In addition we have measured the changes in the level of circulating noradrenaline induced by propranolol

STUDY POPULATION AND METHODS

Fifteen subjects with uncomplicated essential hypertension (mean arterial pressure expressed as diastolic pressure+1/3 pulse pressure ranged between 110 and 130 mmHg) were hospitalized Treatment was discontinued at least 3 weeks before admission Sodium intake was fixed at 60 mmol/day and checked by collection and analysis of 24-hour urine BP was measured at 20 min intervals during the day and overnight using an automatic recording system of indirect BP ("Arteriosonde Roche) It was felt that the use of an invasive technique would interfere with natural behaviour (sleep) whereas acclimatization to the present procedure occurred rapidly after a run in period of one or two nights

For computation of the BP readings we developed the following procedure as proposed by Birkenhager and Schalekamp (5) (Fig 1) The basal BP recorded immediately after waking according to the criterion of Alam and Smirk (1) was used as a reference The maximal excursion in daytime BP was spotted and the difference from basal BP was termed pressor range" The lowest level observed during sleep subtracted from the basal" BP yielded the depressor range The sum of both ranges corresponded to total variability of BP

Samples for determination of plasma renin and plasma noradrenaline concentrations were drawn at 10 a m after overnight recumbency and fasting

Since blood drawn immediately after venipuncture may *contain considerably more noradrenaline than blood taken some time later through an indwelling needle (6 8) we* waited at least 20 min after inserting the needle (18) before blood was collected Plasma renin concentration was determined using the method of Skinner (25) followed by radioimmunoassay of angiotensin I (21) Plasma noradrenaline concentration was measured by a modification of the method described by Henry et al (16) which will be published in detail elsewhere (14)

The studies were undertaken before and after the subjects had been treated with propranolol (160–360 mg daily) for two weeks Statistical analysis was carried out using *Student s paired t test*

RESULTS

Table I presents the individual maximum and minimum BPs before and during treatment

In 10 patients the mean BP measured at 10 a m on the day of study decreased by 15±2 mmHg (mean ±S E M) following propranolol treatment No change was observed in 2 patients and an increase in 3 *Heart rate was reduced in all patients* by an average of 13 beats/min

Pressor range was reduced in 9 patients whilst it was actually increased or unchanged in 6 (Fig 2)

Table I *Maximum and minimum systolic and diastolic BP (mmHg) before (B) and during (D) treatment with propranolol*

Pat no	Systolic max		Systolic min		Diastolic max		Diastolic min	
	B	D	B	D	B	D	B	D
1	210	210	135	160	120	135	95	105
2	175	130	105	100	115	90	65	55
3	150	140	110	100	110	110	75	60
4	205	180	155	140	115	110	75	70
5	250	200	195	165	135	125	120	105
6	170	150	120	115	105	90	80	70
7	205	190	150	140	130	125	105	95
8	175	150	135	110	130	100	100	80
9	165	150	125	115	105	105	75	70
10	*185*	*140*	*120*	*110*	*110*	*95*	*80*	*70*
11	175	160	130	130	120	130	100	100
12	150	110	125	95	100	75	85	70
13	150	140	115	100	105	95	65	60
14	210	225	150	170	120	125	90	95
15	*160*	*125*	*130*	*115*	*110*	*95*	*85*	*80*

Conversely the depressor range i e the nocturnal fall in BP during sleep showed a weak tendency to increase These changes were unrelated to age and response of the 10 a m BP to treatment Total variability of BP remained unchanged during treatment

The mean plasma level of noradrenaline for the whole group before treatment (0 32±0 03 ng/ml) did not differ significantly from that after treatment (0 31±0 03 ng/ml) However when individual changes were considered there was a decrease in 4 patients an increase in 8 and no change in 3

An inverse relationship was observed between changes in plasma noradrenaline levels and pressor range ($r=$ 0 79 $p<0$ 001) (Fig 3) Plasma renin concentration decreased from 8 7±1 4 to 7 6±1 1 ng/ml/h although this change was not significant ($p>0$ 05) In addition there appeared to be no relation between individual changes in plasma renin and pressor range or noradrenaline

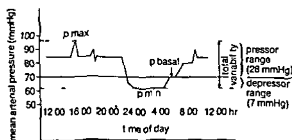


Fig 1 Schematic drawing of diurnal BP fluctuations with proposed quantitative expressions of BP variability

DISCUSSION

This study provides evidence that total variability of BP is not affected by β blockade thus confirming other reports (7 27) We have however further analysed the daily BP pattern in relation to adrenergic activity

While mean noradrenaline levels were not altered during treatment there was considerable individual variation The predominant change was a rise in noradrenaline during β blockade which is in general accordance with other studies (11 19) Acute administration of propranolol does not affect plasma catecholamines (17) unless another stimulus

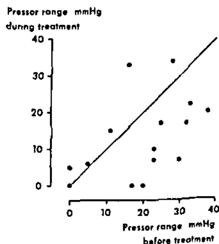


Fig 2 Pressor range before and during treatment with propranolol The line of identity is shown

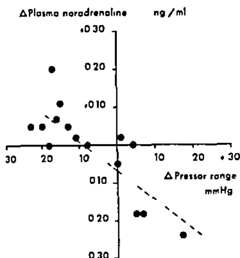


Fig 3 Relation between changes in pressor range and noradrenaline levels in plasma ($r=-0\ 79$ $p<0\ 001$ $y=-0\ 008x-0\ 068$)

(upright posture, exercise) is present (15 17 24) During long term treatment with β blockers plasma noradrenaline either rises (11 19) or stays unaltered (13 24) We have shown here that it can also decrease

The most important feature of the present results is the inverse relationship observed between changes in pressor range (upward variations in BP from basal levels) and changes in plasma noradrenaline levels One explanation of this phenomenon could be an effect of propranolol on (extra)neuronal uptake of noradrenaline in such a way as to distort the relationship between adrenergic nerve traffic and net noradrenaline release However if the above reasoning is correct this should apply to all patients so treated This was not the case in the present study since increased noradrenaline levels were found only in patients who responded with a fall in the pressor range This suggests that the phenomenon has a true physiological meaning The pressor range being a quantitative expression of daytime lability of BP could depend both on variations in myocardial contractility and in arteriolar tone Since changes in pressor range occurred independently of the reduction in heart rate (and therefore presumably myocardial contractility) alterations in vessel wall tone are likely to be the main determinant

One could think of the renin–angiotensin system as mediator of the altered vessel tone Although renin levels have been shown to be related to catecholamines in the basal state (9 10) a dissociation between the two variables occurs during β blocker therapy (11 13) While there is little doubt that renin levels are lowered by β blockers it has been suggested that insufficient reduction of renin in some patients can evoke pressor responses (12) However in our study, renin levels were reduced to the same extent irrespective of changes in the pressor range indicating that the renin–angiotensin system probably does not play an important role in such alterations in arteriolar reactivity

The finding that the fall in pressor range occurs in the face of increased noradrenaline levels is rather surprising This may indicate that noradrenaline secretion is stimulated or inhibited to compensate for alterations in vessel wall tone induced by another mechanism which remains to be identified

ACKNOWLEDGEMENTS

The investigation was supported by grant GO 732-61 from the Organization for Health Research T N O the Hague and a combined grant from the Organization of Health Research T N O and the Foundation of Medical Research Z W O Rijswijk the Netherlands

REFERENCES

1 Alam G M & Smirk F H Br Heart J 5 152, 1943
2 Athanassiadis D Draper G J Honour A J & Cranston W I Clin Sci 36 147 1969
3 Bevan A T Honour A J & Stott F H Clin Sci 36 329 1969
4 Birkenhäger W H Van Es L A Houwing A Lamers H J & Mulder A H Clin Sci 35 445 1968
5 Birkenhäger W H & Schalekamp M A D H *Control mechanisms in essential hypertension* p 11 Elsevier/North Holland Biomedical Press Amsterdam 1976
6 Carruthers M Taggart P Conway N Bates D & Somerville W Lancet 2 62 1970
7 Clement D L Bogaert M G & Pannier R Abstract book II of the seventh European Congress of Cardiology p 83 Amsterdam 1976
8 Cryer P E Santiago J V & Shah S J Clin Endocrinol Metab 39 1025 1974
9 De Quattro V Campese V Miura Y & Esler M Clin Sci Mol Med 51 435s 1976
10 De Quattro V & Miura Y Am J Med 55 362 1973
11 Distler A Cordes U Walter U & Philipp Th Therapiewoche 26 7542 1976
12 Drayer, J I M Keim H J Weber M A Case B & Laragh J H Am J Med 60 897

13 Esler M Julius S Zweifler A, Randall O Harburg E Gardiner H & De Quattro V N Engl J Med 296 405 1977
14 Falke H E Submitted to Clin Chim Acta 1977
15 Galbo H Holst J J Christensen N J & Hilsted J J Appl Physiol 40 855 1976
16 Henry D P Starman B J Johnson D G & Williams R H Life Sci 16 375 1975
17 Irving M H Britton B J & Wood, W G Nature 248 531 1974
18 Lake C R Ziegler M G & Kopin I J Life Sci 18 1315 1976
19 Rahn K H Gierlichs H W Planz G Planz R & Stephany W Eur J Clin Invest 6 323 1976
20 Richardson D W Honour A J Fenton G W Stott F H & Pickering G W Clin Sci 26 445, 1964
21 Schalekamp M A D H Schalekamp Kuyken M P A De Moor Fruytier M Meininger Th Vaandrager Kranenburg D J & Birkenhäger W H Clin Sci 45 417 1973
22 Schneider R A J Appl Physiol 24 115 1968
23 Shaw D B Knapp M S & Davies D H Lancet 1 797 1963
24 Simrock R Appel E Palm D Grobecker H Philipp, Th & Distler A Therapiewoche 26 7536 1976
25 Skinner S L Circ Res 20 391 1967
26 Sokolow M Werdegar D Kain H K & Hinman A T Circulation 34 279 1966
27 West M J Honour A J, Sleight P & Littler W A Aust NZ J Med (Suppl) 3 19 1976

Acta Med Scand 202 393–397 1977

Glucose Tolerance and Insulin Release in Hypertensive Patients Treated with the Cardioselective β-Receptor Blocking Agent Metoprolol

Goran Ekberg and Bengt-Goran Hansson

From the Department of Endocrinology University of Lund Malmo General Hospital Malmo Sweden

ABSTRACT Blood glucose and plasma insulin levels were studied under fasting conditions and following an i v and an oral glucose load, respectively in nine males with moderate hypertension before treatment after one month on placebo and after three months on the cardioselective β receptor blocking agent metoprolol The studies were performed under metabolic ward conditions The reproducibility of blood glucose and plasma insulin values following an i v glucose load was very good Medication with metoprolol caused no changes in the fasting levels of blood glucose or plasma insulin, nor in the blood glucose response following a glucose load given i v or orally The initial and total integrated insulin response to the i v administration of glucose was similar before and during metroprolol Following oral glucose both the total integrated blood glucose response and the insulin response were unaffected by treatment with metoprolol

Adrenergic mechanisms can modify blood glucose levels and glucose induced insulin release in man (3 5 16 18 19 20 21) Studies on the non selective β receptor blocking agent propranolol have however given conflicting results on both insulin secretion and glucose metabolism (2 3 6 10 21 22 24) Recently Hansson and Hökfelt (8) reported that the non selective β receptor blocking agent penbutolol had no effect on blood glucose and insulin levels under fasting conditions nor on the glucose and insulin response following glucose given i v or orally In the present investigation the same parameters were studied before and during treatment with the cardioselective β receptor blocking agent metoprolol

PATIENTS AND METHODS

The patients were nine males with moderate hypertension (Table I) Seven were considered to have essential hypertension one had polycystic renal disease and one had remarkably low urinary aldosterone excretion None had known family history of diabetes mellitus All had normal fasting blood glucose levels and the glucose disappearance rate (k value) following an i v glucose load was normal in all

During the studies the patients were hospitalized in a metabolic ward and were given a diet containing 120 mEq sodium and 95 mEq potassium daily All studies were performed in an identical manner before treatment after one month on placebo and after about three months on metoprolol During the studies no medication except metoprolol was permitted Metoprolol (Seloken® Hassle) 150–450 mg daily was given orally in divided dosages at 7 a m 2 p m and 10 p m In all patients treatment with metoprolol resulted in significant decreases in BP pulse rate and plasma renin activity (7)

At 10 p m on the day before each glucose tolerance test 55 g glucose (corresponding to 50 g water free glucose) was given orally dissolved in water The patients were then fasting and they were confined to bed until the end of the test

For the i v glucose tolerance test 25 g glucose/m² BSA was injected i v as a 40% solution during 5 min starting at 7 30 a m Samples for determination of blood glucose were drawn from an antecubital vein immediately before

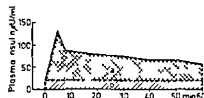


Fig 1 Integrated insulin (glucose) response Area a = integrated response measured from initial level Areas $a+b$ = integrated response measured from zero level

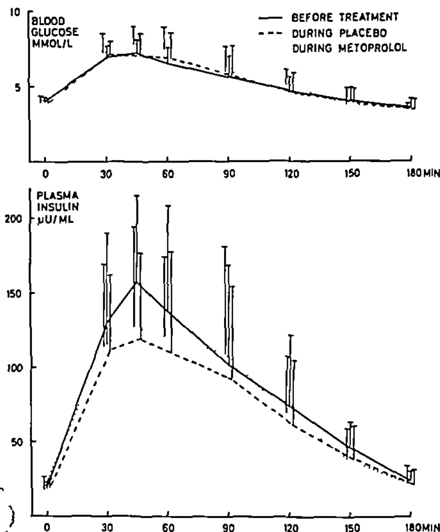


Fig 2 Blood glucose and plasma insulin levels (mean ± S D) before treatment during placebo and during metoprolol following an oral glucose load

the glucose injection and then at 10 20 30 40 50 and 60 min after the start of injection For the assay of plasma insulin (determined by J I Thorell) blood was drawn immediately before the injection of glucose and then at 5 8 20 30 40 50 and 60 min after the start of the injection

For the oral glucose tolerance test 30 g glucose/m² BSA dissolved in water was given at 7 00 a m Blood samples for determination of glucose and insulin were drawn from an antecubital vein immediately before the glucose load and after 30 45 60 90 120 150 and 180 min

Blood glucose was determined using a glucose oxidase method (13 15) Plasma insulin was measured in triplicate using radioimmunoassay (9) All series of blood samples for determination of plasma insulin were analyzed in one and the same single assay run for each patient

Statistical significance of differences was assessed with Student s *t* test for paired data Significance was recorded for $p<0\ 05$

The insulin response to glucose given i v or orally was calculated in different ways (see below) The principles for calculation of the integrated glucose and insulin response are presented in Fig 1

Table I *Some clinical and laboratory data on the patients before medication with metoprolol*

Pat no	Age (y)	B wt (kg)	B ht (cm)	Mean supine BP[a]	Serum creatinine (µmol/l)	*K* value (%/min)
1	47	74	175	155/103	110	1 3
2	38	72	167	140/101	100	1 7
3	31	91	173	142/107	120	1 3
4	44	82	173	154/111	100	1 4
5	38	85	169	136/96	110	1 0
6	51	89	176	145/103	90	1 4
7	45	74	162	148/101	110	1 6
8	50	74	173	152/93	100	0 9
9	52	87	175	152/108	100	1 0

[a] Measured during hospitalization

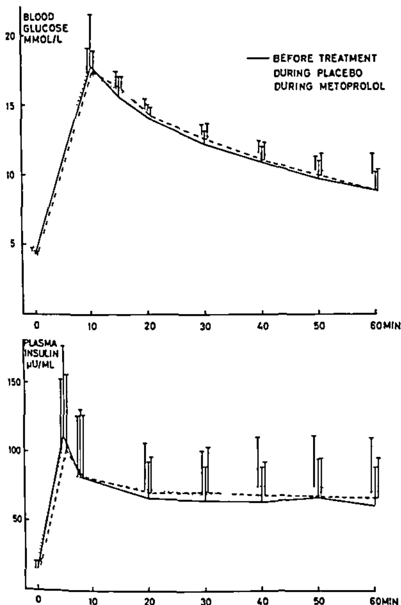


Fig 3 Blood glucose and plasma insulin levels (mean ± S D) before treatment during placebo and during metoprolol following an i v glucose load

RESULTS

No significant changes in fasting blood glucose and plasma insulin occurred during placebo nor was there any change in the response of glucose and insulin to an i v or oral glucose load ($p>0\ 05$) (Figs 2 and 3) In the individual patient the absolute values for blood glucose and plasma insulin measured at each time of sampling after an i v glucose load showed very small variations before treatment compared with the values during placebo although wide variations were seen between patients The reproducibility of blood glucose and plasma insulin values in connection with an oral glucose load was satisfactory but not as good as in connection with an i v glucose load

Metoprolol caused no changes in fasting glucose ($p>0\ 05$) or fasting plasma insulin levels ($p>0\ 05$) (Figs 2 and 3) nor did it alter either the maximal rise in blood glucose after an i v glucose challenge ($p>0\ 05$) (Fig 2) or the glucose disappearance rate expressed as the integrated blood glucose response during 60 min ($p>0\ 05$) or as k value ($p>0\ 05$)

The i v injection of glucose induced a marked and identical increase in plasma insulin before and

during treatment with metoprolol (Fig 2) The early insulin response as measured at 5 and 8 min after the start of the glucose injection was of the same magnitude before and during treatment with metoprolol ($p>0$ 05) The integrated insulin response measured both from initial and zero values was unaffected by metoprolol The ratio between integrated blood glucose response and integrated insulin response measured either from initial or from zero values was not altered by metoprolol ($p>0$ 05)

Oral administration of glucose led to a similar increase in blood glucose levels before and during metoprolol ($p>0$ 05) (Fig 3) Metoprolol did not affect the glucose induced insulin release after oral glucose (Fig 3) calculated as the integrated insulin response during 180 min measured either from the initial insulin levels or from zero levels ($p>0$ 05) Again metoprolol had no influence on the ratio between integrated blood glucose response and integrated insulin response ($p>0$ 05)

DISCUSSION

In contrast to a previous report (11) the reproducibility of the glucose and insulin response following an i v glucose load was very good in the present studies This was probably due to the fact that they were performed under well defined conditions ... ided this is the case the i v glucose tolerance test can be used to study the influence of various factors such as drugs on glucose tolerance and glucose induced insulin response Also the oral glucose tolerance test showed reasonably good reproducibility in our study, again in contrast to earlier results (14)

Stimulation of α and β receptors can affect insulin release The infusion of adrenaline (19) as well as noradrenaline (20) has been found to inhibit pancreatic insulin release, and patients with phaeochromocytoma have been reported to show a decreased insulin response following a glucose load (4 12, 23 25 26) This inhibition of insulin secretion has been attributed to stimulation of α receptors (17) because α receptor blockade increases both basal (5 6 16 21) and glucose induced insulin secretion (5)

Adrenergic β receptor stimulation with isoproterenol in the normal individual has been reported to increase insulin secretion (18) but whether this increase is mediated via β_1 or β_2 receptors is not clear Acute treatment with propranolol has given conflicting results i e decreased unchanged or increased insulin secretion (2 3 6 10 21 22 24) Similarly studies on the effect of propranolol on glucose tolerance test have given variable results (1 6) Long term treatment with propranolol (10) and penbutolol (8) caused no difference in the insulin response following an i v glucose load in man

No change in carbohydrate tolerance was observed in our studies during long term treatment with metoprolol There was no evidence of decreased insulin production during medication with respect to either the initial or the late glucose induced insulin response Thus, our results with metoprolol are similar to earlier results during long term treatment with non selective β receptor blocking agents Our results support earlier findings that the insulin response to glucose in non diabetic subjects is mediated by specific pancreatic glucose receptors which are independent of β_1 adrenergic receptors

ACKNOWLEDGEMENTS

The studies were supported by grants from the Swedish Society of Medical Sciences and AB Hassle Molndal Sweden

REFERENCES

1 Abramson E A Arky R A & Woeber K A Effects of propranolol on the hormonal and metabolic responses to insulin induced hypoglycemia Lancet 2 1386 1966
2 Allison S P Chamberlain M J Miller J E Ferguson R Gillett A P Bemand B V & Saunders R A Effects of propranolol on blood sugar insulin and free fatty acids Diabetologia 5 339 1969
3 Cerasi E Luft R & Efendić S Effect of adrenergic blocking agents on insulin response to glucose infusion in man Acta Endocrinol 69 335 1972
4 Colwell J A Inhibition of insulin secretion by catecholamines in pheochromocytoma Ann Intern Med 71 251 1969
5 Cryer P E Herman C M & Sode J Insulin release during alpha adrenergic receptor blockade Primacy of the glycemic stimulus Endocrinology 89 918 1971
6 Gagliardino J J Bellone C F Doria I Sanchez J J & Pereyra V Adrenergic regulation of basal serum glucose NEFA and insulin levels Horm Metab Res 2 318 1970
7 Hansson B -G Dymling J F Hedeland H & Hulthen U L Long term treatment of moderate hypertension with the beta$_1$ receptor blocking agent metoprolol I Effect on maximal working capacity

and on plasma catecholamines plasma renin activity urinary aldosterone blood pressure and pulse rate under basal conditions Accepted for publication in Eur J Clin Pharmacol 1977

8 Hansson B-G & Hokfelt B Long term treatment of moderate hypertension with penbutolol (Hoe 893d) III Effects on glucose tolerance and insulin production Eur J Clin Pharmacol 10 157 1976

9 Heding L G A simplified insulin radioimmunoassay method In Labelled proteins in tracer studies (ed L Donato G Milhaud & J Sirenis) p 345 Euratom Brussels 1966

10 Hedstrand H & Åberg H Insulin response to intravenous glucose during long term treatment with propranolol Acta Med Scand 196 39 1974

11 Hedstrand H & Boberg J Statistical analysis of the reproducibility of the intravenous glucose tolerance test and the serum insulin response to this test in middle aged men Scand J Clin Lab Invest 35 331 1975

12 Illig R & Ziegler W H Glucose tolerance and immunoreactive insulin in patients with pheochromocytoma. The effect of alpha receptor blocking agents Acta Endocrinol 66 368 1971

13 Marks V An improved glucose oxidase method for determining blood C S F and urine glucose levels Clin Chim Acta 4 395 1959

14 McDonald G W Burnham C E & Lewis W F Reproducibility of glucose tolerance in 101 non diabetic women Public Health Rep (Wash) 84 353 1969

15 Middleton J E & Griffiths W J Rapid colorimetric micromethod for estimating glucose in blood and C S F using glucose oxidase Br Med J II 1525 1957

16 Persson I & Larsen L Serum insulin concentration after alpha adrenergic blockade and secretin in diabetics Acta Endocrinol 71 331 1972

17 Porte D A receptor mechanism for the inhibition of insulin release by epinephrine in man J Clin Invest 46 86 1967

18 Porte D Jr Beta adrenergic stimulation of insulin release in man Diabetes 16 150 1967

19 Porte D Jr Graber A L Kuzuya T & Williams R H The effect of epinephrine on immunoreactive insulin levels in man J Clin Invest 45 228 1966

20 Porte D Jr & Williams R H Inhibition of insulin release by norepinephrine in man Science 152 1248, 1966

21 Robertson R P & Porte D Jr Adrenergic modulation of basal insulin secretion in man Diabetes 22 1 1973

22 — The glucose receptor A defective mechanism in diabetes mellitus distinct from the beta adrenergic receptor J Clin Invest 52 870 1973

23 Spergel G Bleicher S J & Ertel N H Carbohydrate and fat metabolism in patients with pheochromocytoma N Engl J Med 278 803 1968

24 Sussman K E Stjernholm M R & Vaughan G D Propranolol and hypoglycaemia Lancet 1 626 1967

25 Vance S E Buchanan K D O Hara D Williams R H & Porte D Jr Insulin and glucagon responses in subjects with pheochromocytoma Effect of alpha adrenergic blockade J Clin Endocrinol 29 911 1969

26 Wilber J F Turtle J R & Crane N A Inhibition of insulin secretion by a phaeochromocytoma Lancet 1 733 1966

Acta Med Scand 202 399–408 1977

Lipoprotein Lipase Activity in Adipose Tissue and in Postheparin Plasma in Human Obesity

Marja Riitta Taskinen and Esko A Nikkila

From Third Department of Medicine University of Helsinki Helsinki Finland

ABSTRACT The mechanism by which obesity influences the metabolism of circulating triglycerides was assessed by determination of lipoprotein lipase (LPL) activity in adipose tissue and in postheparin plasma of 26 normolipidemic obese and 26 normal weight subjects Furthermore, the uptakes of VLDL triglyceride and FFA by adipose tissue in vitro were measured in these two groups The heparin releasable LPL activity per weight unit of adipose tissue was similar in obese and normal weight subjects but the LPL activity per fat cell was on an average twice as high in obese as in non obese subjects Also the VLDL triglyceride and FFA uptakes by adipose tissue were similar in obese and non-obese groups when related to tissue weight but both were increased in obese fat cells compared with those of non-obese When expressed per total body fat all these parameters of adipose tissue lipid uptake were higher in obese than in normal weight subjects In contrast, the obese group did not show any increase in the postheparin plasma LPL activity but rather a tendency for the values to accumulate in the low normal range Serum triglyceride concentration was not related to any of the LPL activities or to tissue lipid uptake in separate or combined obese and non-obese groups Serum basal insulin level and insulin response to glucose were significantly higher in the obese than in non obese subjects but in spite of this, the serum triglyceride level was similar in the two groups Stepwise multiple regression analysis showed that all the measured parameters of adipose tissue were positively correlated to fat cell size but when the latter was held constant, the fat cell VLDL triglyceride uptake was still related to cell LPL activity and to serum insulin concentration These results suggest that in obesity the adipose tissue has an increased capacity to remove circulating triglycerides

Serum triglyceride and very low density lipoprotein (VLDL) levels are increased in obese co red with normal weight subjects (2 14 23) The reasons for this metabolic abnormality are not clear but some studies suggest that the hepatic production of VLDL triglycerides is increased in both experimental (3 35 38) and human (21 29) obesity The accelerated production of VLDL in obesity might be a consequence of an increased turnover of plasma free fatty acids (FFA) (25) or of an augmented synthesis of hepatic secretory triglycerides from non FFA precursors (4)

The question whether obesity might impair the removal of plasma endogenous triglycerides is so far unanswered In genetically obese mice the clearance of labeled plasma triglyceride and of Intralipid® are both increased compared with lean mice (38) In man the fractional removal of Intralipid is independent of relative body weight when the influence of the latter on plasma triglyceride concentration is taken into account (36) The authors of this study believe however that in relation to the amount of adipose tissue the triglyceride removal sites are decreased in obesity and that there might be a relative impairment of removal (36) A decreased lipoprotein lipase (LPL) activity of adipose tissue has in fact been reported in both human (31) and experimental (24) obesity However all of the more recent studies have convincingly shown that the adipose tissue LPL activity is positively correlated to fat cell size in animals (10 34) and in man (5 6 13 20 32) Since adult-onset obesity is usually accompanied by enlargement of adipocytes (40) the total adipose tissue LPL activity of obese people is expected to be high rather than low

The removal of VLDL triglycerides occurs not only in adipose tissue but also in skeletal muscle and myocardium where LPL also acts as a key

enzyme The sum of all tissue LPL activities is best reflected by postheparin plasma LPL activity as sayed by a selective method In normal human subjects the fractional removal rate of VLDL triglycerides is positively correlated to postheparin plasma LPL activity (18) On the other hand the postheparin plasma LPL of non obese subjects shows an inverse correlation to body weight (18)

Since the assay of postheparin plasma LPL offers a new approach to the elucidation of the mechanism of obesity induced increase in serum triglyceride concentration we have measured the LPL in adipose tissue and postheparin plasma of obese normolipidemic subjects In addition the uptakes of VLDL triglyceride and FFA by adipose tissue in vitro were studied

STUDY POPULATION AND METHODS

Subjects Fifty two subjects were studied 26 classified as obese and 26 as of normal weight Obesity was considered to be present when the relative body weight (Geigy Tables) exceeded 120% There were 5 males and 21 females in the obese group age range 18–54 years (mean 37±2) and 8 males and 18 females in the normal weight group age range 22–64 years (mean 36±2)

Serum triglyceride level was below 1 8 mM and fasting blood glucose level below 5 0 mM in all subjects Thus patients with hypertriglyceridemia or with clinical diabetes were excluded from the present material

Sampling of adipose tissue Specimens were taken from subcutaneous adipose tissue by surgical biopsy In 41 cases the biopsy specimen was taken in connection with abdominal surgery (cholecystectomy herniorrhaphy) in 11 cases under local anesthesia (Lidocain®) from an incision of 2–3 cm In all instances the patients had been fasted overnight at the time of the biopsy The specimen weighing c 500 mg was placed in 0 9% saline and thereafter divided into pieces weighing 10–20 mg which were used for different assays

Adipose tissue lipoprotein lipase activity The assay system included two separate incubations In the first the LPL was released from the tissue into the medium with heparin elution and in the second the eluted enzyme was incubated with substrate (VLDL) in the absence of tissue Thus the assay system measured only the heparin releasable part of the total adipose tissue LPL activity It has been shown previously however that there is an excellent correlation between the total and heparin releasable LPL acitivites of human adipose tissue (33)

Pieces of adipose tissue (20–50 mg) were incubated in duplicate vials containing 1 4 ml of 0 1 M Krebs Ringer Tris buffer (pH 8 1) with 4 g/100 ml of bovine serum albumin 0 5 ml of pooled human serum (with triglyceride concentration of 2 0 mM) and 0 1 ml (50 μg) of heparin (Vitrum Sweden) Incubation was carried out at 37°C in a metabolic incubator with slight agitation After 20 min the tissue pieces were removed from the medium and the substrate was added in a volume of 10–50 μl to give a radioactivity of 4000–6000 cpm in the total incubation mixture Samples were removed from the medium for assay of radioactivity and of triglyceride concentration Incubation was then continued for 60 min at 37°C after which 1 ml of the medium was taken into 10 ml of Dole s solution The mixture was shaken for 10 min and the phases were separated by adding heptane and water The heptane phase was separated after 10 min extraction and the lower phase was washed once with heptane The combined heptane phases were saponified with 10 ml of 0 05 N NaOH in 50% ethanol The separated alkaline ethanol phase was washed with heptane and the combined heptane phases were transferred to a Packard counting vial and taken to dryness (substrate radioactivity) The alkaline ethanol phase was acidified with 1 4 ml of 1 N sulphuric acid and the fatty acids were taken into 10 ml of heptane This was again transferred to a Packard counting vial and taken to dryness (product FFA radioactivity) All radioactivities were measured in a Packard Tri Carb® model 547 liquid scintillation counter using PPO toluene scintillation liquid The medium taken before the second incubation did not contain any radioactivity in the FFA fraction The recovery of the medium radioactivity at the end of incubation was 97±2%

Rat VLDL labeled in vivo with radioactive palmitate was used as substrate This was prepared weekly as follows Fifty μCi of palmitic acid 1 ^{14}C (sp a 59 mCi/mmole Radiochemical Centre Amersham England) in benzene were evaporated to dryness and dissolved with hot 0 2 N KOH The solution was mixed with 1 ml of saline containing 50 mg of bovine albumin and then injected into a tail vein of a fed rat The animal was exsanguinated 30 min later serum was separated and VLDL was obtained by ultracentrifugation at a density of 1 006 for 16 hours The flotated VLDL fraction was sliced in a volume of 1 0 ml and used as described above More than 90% of the VLDL radioactivity was present in triglyceride moiety The LPL activity of adipose tissue was calculated from the following formula

$$\text{LPL (nmoles FFA h}^{-1}\text{ g}^{-1}) = \frac{A \quad D \quad 10^6}{B \quad C}$$

where A = medium FFA radioactivity at the end of incubation B = medium substrate radioactivity at the start of incubation C = weight of the tissue (mg) D = medium triglyceride (μmoles) The LPL activity per fat cell was obtained by dividing the activity per gram by the number of fat cells in one gram of the tissue

Incorporation of VLDL TGFA into adipose tissue in vitro Pieces of adipose tissue weighing 50–100 mg per vial were incubated in triplicate in a medium containing Krebs Ringer bicarbonate (KRB) buffer (pH 7 4) 4 g/100 ml of bovine serum albumin 5 mM glucose 1 0 mM of triglyceride as Intralipid® and palmitic acid 1 ^{14}C labeled rat VLDL-TG (4000–6000 cpm) in a final volume of 2 0 ml At the start of the incubation 100 μl of the medium was removed for assay of triglyceride concentration The incubation was made at 37°C in O_2–CO_2 atmosphere with gentle agitation After 60 min the tissue pieces were carefully removed and rinsed with 10 ml of albumin KRB buffer and thereafter with 100 ml of saline The rinsed

pieces were then homogenized with an Ultra Turrax homogenizer into 10 ml of Dole s solution and agitated for 60 min Thereafter the phases were separated by adding heptane and water and shaking for a further 10 min The heptane phase was transferred into a Packard counting vial the water phase was washed once with heptane and the washing phase was added to the counting vial The heptane was evaporated to dryness PPO toluene scintilla tion solution was added and the radioactivity was meas ured in a Packard scintillation counter

Each series included two vials which were incubated for 2 min and the tissue processed as described above These samples served as indicators of the efficiency of the rinsing procedure and the results of any series were ac cepted only when the tissue radioactivity in these control incubations was less than 5% of the value obtained at 60 min The recovery of radioactivity in the tissue plus medium at the end of incubation was 96±1 4% Of the medium radioactivity 0 5–1 0% was present as FFA at the end of the incubation

The VLDL TGFA uptake by adipose tissue was calcu lated from the following formula

$$\text{TGFA uptake (nmoles h}^{-1}\text{ g}^{-1}) = \frac{A \quad D \quad 10^6}{B \quad C}$$

where A = tissue triglyceride radioactivity at 60 min B medium TGFA radioactivity at the start of incubation C tissue weight (mg) D = medium triglyceride (μmoles) The TGFA uptake per fat cell was calculated by dividing the value per gram by the number of fat cells in one gram of tissue

Incorporation of palmitic acid into adipose tissue in vitro Pieces of adipose tissue weighing 50–70 mg per vial were incubated in triplicate in a medium containing KRB buffer (pH 7 4) 4 g/100 ml of bovine serum albumin and 5 mM of glucose in a total volume of 2 0 ml Palmitate 1 ^{14}C was prepared as described above and added to the medium in a volume of 5–10 μl to give 5 000–6 000 cpm A 100 μl sample of the medium was removed for assay of FFA concentration and the incubation was carried out under conditions similar to those described in VLDL TGFA uptake determination After 60 min incubation the tissue pieces were rinsed and processed as indicated above Two minute incubations were used as a check of contamination The recovery of the radioactivity at the end of incubation was 102±4% Uptake of palmitic acid was calculated by the formula given for VLDL TGFA uptake except that B radioactivity of added palmitic acid and D = medium FFA (μmoles)

Fat cell si e Fat cells were released from adipose tissue pieces by collagenase treatment The cells were separated from residual tissue by filtration through a nylon gauze with 200 μm mesh size The adipocytes were washed with saline and two 10 μl aliquots were transferred into a siliconized counting chamber with 0 2 mm depth The fat cell diameter was determined with an ocular micrometer from 250–500 cells The mean diameter was taken to rep resent the fat cell population in each sample and the fat cell weight was calculated by an IBM 1800 computer

Lipoprotein lipase activity of postheparin plasma After an overnight fast the patients received 1 mg of heparin/kg b wt (Vitrum Sweden) as an i v bolus injection Blood was drawn 15 min later from the contralateral cubital vein into tubes held in ice Plasma was separated by centrifuga tion in cold and frozen LPL activity was determined within two weeks by a selective immunochemical method (19) which separates the LPL from the hepatic triglyceride lipase activity The heparin test was always carried out one or more days after the adipose tissue biopsy in order to avoid the possible error due to depletion of the enzyme activity in tissues by previous heparin administration

Oral glucose tolerance test Glucose 1 g/kg b wt was given orally as a 20% solution after a 12 hour overnight fast Blood glucose and serum insulin (15) were de termined at 0 1 2 and 3 hours

Other measurements Cholesterol (16) triglyceride (22) and FFA (27) concentrations in serum or incubation media were measured by standard procedures Total body fat mass was estimated from weight and height according to Hume (17) by subtracting the calculated lean body mass from absolute weight

Statistical methods The average error of one incuba tion was calculated by a standard computer program made for the IBM Nova computer The percentile errors for adipose tissue LPL VLDL TGFA uptake and palmitic acid uptake were 8 8% 12 5% and 14 1% respectively The multiple correlations were calculated by standard multiple regression computer analysis with IBM Nova (1)

RESULTS

Characteristics of the adipose tissue morphology of obese and non obese groups are shown in Table I Both the total body adipose tissue mass and the individual mean fat cell size were increased twofold

Table I *Amount and composition of adipose tissue (mean ± S E M)*
RBW = relative body weight

Subjects	RBW (%)	Body fat (kg)	Fat cell diameter (μm)	Fat cell size (μg)	Fat cell number (×10¹⁰)
Non-obese (n=26)	105±2	17 9±1 0	80±2 7	0 31±0 03	6 22±0 38
Obese (n=26)	154±8	36 3±2 4**	105±3 3*	0 69±0 04**	5 56±0 32

p<0 01

Table II *Basic metabolic parameters (mean ± S E M)*

Subjects	Glucose (mM)	Insulin (μU/ml)	ΣΔ insulin[a]	Triglyceride (mM)	Cholesterol (mM)
Non-obese	3 8±0 1	9 0±0 9	52±7	0 99±0 06	5 5±0 3
n	26	19	18	26	26
Obese	4 0±0 1	20 9±1 9**	177±40**	1 09±0 07	5 6±0 3
n	26	20	18	26	26

[a] Incremental insulin values measured at 1 2 and 3 hours during an oral glucose tolerance test
** $p<0\ 01$

Table III *Heparin releasable lipoprotein lipase activity of adipose tissue (mean ± S E M)*

Subjects	Lipoprotein lipase activity (FFA h⁻¹)		
	Per g (nmoles)	Per cell ×10⁶ (nmoles)	Per total body fat (mmoles)
Non-obese (n=26)	696±280	204±30	11 7±1 4
Obese (n=26)	760±180	567±70***	28 2±3 9***

*** $p<0\ 001$

in obese subjects compared with non obese whereas no significant difference was found in average fat cell number Thus in the present material obesity was mainly due to enlargement of fat cells The average serum triglyceride and cholesterol concentrations were similar in the two groups (Table II) On the other hand the basal serum insulin level and the mean insulin response to oral glucose were markedly increased in the obese group (Table II)

Adipose tissue lipoprotein lipase activity

The LPL activity expressed per adipose tissue weight was similar in obese and non obese subjects (Table III) However, the average LPL activity of a fat cell was 2 8 times and the total body adipose tissue LPL activity 2 4 times higher in obese than in normal weight subjects (Table III)

The mean fat cell LPL activity was positively related to fat cell size (Fig 1) to basal serum insulin concentration (Fig 2 Table IV) and to insulin response to glucose ($p<0\ 05$) Since the fat cell size on the other hand was related to serum insulin level partial correlations were calculated to estimate whether serum insulin affected the fat cell LPL activity directly When fat cell size was kept constant the correlation between serum basal insulin and fat cell LPL was lost ($r=0\ 22$ $p>0\ 1$)

There was no significant correlation between adipose tissue LPL activity and serum triglyceride concentration (or VLDL triglyceride level) in sepa

Table IV *Correlation coefficients (r) and their significance (p)*

x	*y*	*n*	*r*	*p*
Fat cell size	Relative b wt	52	0 78	<0 001
	Basal serum insulin	36	0 59	<0 001
	AT LPL activity per cell	52	0 82	<0 001
	VLDL TGFA uptake per cell	36	0 72	<0 001
	Palmitic acid uptake per cell	36	0 57	<0 001
Serum insulin	AT LPL activity per cell	35	0 48	<0 01
	VLDL-TGFA uptake per cell	22	0 66	<0 001
	Palmitic acid uptake per cell	23	0 27	NS
AT LPL activity per cell	Serum triglyceride	52	−0 04	NS
	PH LPL activity	32	−0 33	NS
	VLDL TGFA uptake per cell	36	0 79	<0 001
	Palmitic acid uptake per cell	36	0 73	<0 001
Total adipose tissue LPL activity	Serum triglyceride	52	−0 02	NS
	PH LPL activity	36	−0 32	NS
VLDL TGFA uptake	Palmitic acid uptake	36	0 52	<0 001

NS=not significant

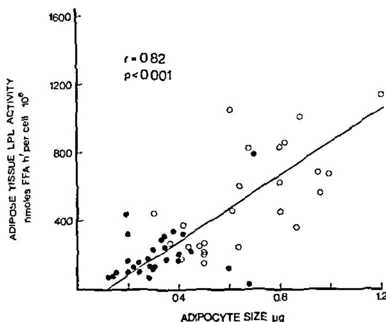


Fig 1 Correlation between mean fat cell lipoprotein lipase (*LPL*) activity and fat cell size in obese (O) and non-obese (●) subjects

rate or combined obese and non obese groups This correlation was absent irrespective of whether the LPL activity was expressed per adipose tissue weight per fat cell or per total body fat or whether the logarithm of triglyceride was used (*Table IV*)

Postheparin plasma lipoprotein lipase activity

The individual values of postheparin plasma LPL activity are shown in Fig 3 Although the postheparin LPL activity in many of the obese subjects was within the lower range of the values of non-obese controls the wide scatter of the values in both groups made that the difference between the mean values (22 9±5 7 vs 26 1±5 3 μmol FFA h^{-1} ml^{-1} for obese and non obese respectively) was not significant

The postheparin plasma LPL and adipose tissue LPL activities did not show a significant correlation with each other either in the whole material or in the normal weight and obese groups separately (Table IV) However the obese group showed a tendency to an inverse correlation between the two LPL activities

Uptake of VLDL TGFA and of free palmitic acid by adipose tissue in vitro

The incorporation of VLDL triglyceride fatty acid and that of FFA into adipose tissue triglyceride in vitro were similar in obese and non obese groups when expressed per tissue weight Again when the uptake is given per fat cell the obese subjects have higher incorporation rates of both substrates than the normal weight individuals (Table V) The uptake of both substrates was related to fat cell size (Table IV)

Both the VLDL TGFA and the palmitic acid up

Table V *In vitro incorporation (nmoles h^{-1}) of VLDL TGFA and of palmitic acid into adipose tissue triglyceride (mean ± S E M)*

Subjects	Incorporation of VLDL TGFA		Incorporation of palmitic acid	
	Per g	Per cell ×10⁶	Per g	Per cell ×10⁶
Non-obese (n=18)	686±60	206±39	244±31	75±11
Obese (n=18)	598±46	413±57 *	176±26	127±27*

*p<0 05 **p<0 01

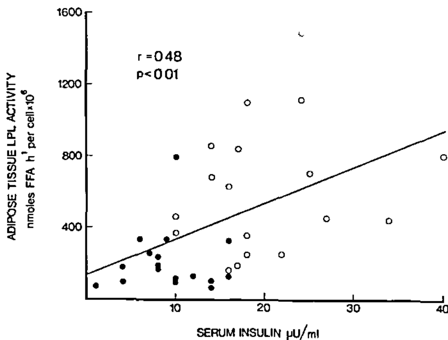


Fig 2 Correlation between mean fat cell lipoprotein lipase (*LPL*) activity and basal serum insulin in obese (O) and non-obese (●) subjects

take were significantly correlated to the heparin-elutable LPL activity of adipose tissue (Figs 4 and 5) Since all three parameters were on the other hand closely related to fat cell size partial correlation coefficients were calculated using a constant fat cell size Even with this procedure the correlation between VLDL-TGFA uptake and LPL activity ($r=0.42$ $p<0.01$) as well as that between FFA uptake and LPL activity ($r=0.56$ $p<0.01$) remained significant indicating that the relationships were apparently direct and not necessarily caused by fat cell size as such

The VLDL-TGFA uptake by adipose tissue was related to basal serum insulin level but the FFA uptake was not (Table IV) The former correlation remained significant ($r=0.53$ $p<0.01$) when the influence of fat cell size was eliminated

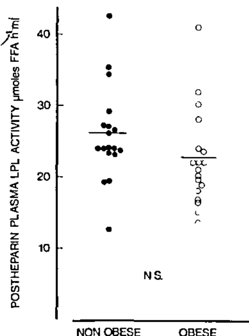


Fig 3 Individual values of postheparin plasma lipoprotein lipase activity in obese (O) and non obese (●) subjects after a 12 hour overnight fast The means are indicated by horizontal lines

DISCUSSION

A slight to moderate elevation of the serum triglyceride (and VLDL) level is more common in obese than in non obese people (2 14) and overweight is one of the few factors known to be associated with hypertriglyceridemia It is therefore evident that obesity either increases the rate of secretion of triglycerides into the plasma or impairs their removal from the circulation Simple overfeeding of normal weight people does not lead to a constant elevation of the serum triglyceride level (17 28 39) indicating that the disturbance of plasma triglyceride metabolism requires the accumulation

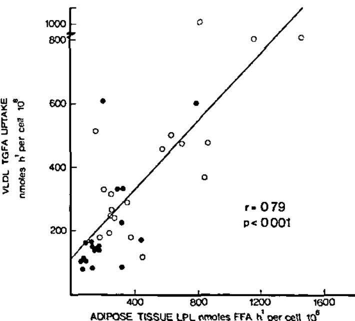


Fig 4 Correlation between VLDL TGFA uptake expressed per fat cell and mean fat cell lipoprotein lipase (*LPL*) activity in obese (O) and non-obese (●) subjects

of a certain amount of adipose tissue or that obese people have some basic abnormality which favors the triglyceride synthesis both in adipose tissue and in the liver An increase of hepatic plasma triglyceride production has been demonstrated in experimental obesity of animals but is not a consistent finding in obese man (21 29)

Since a true familial or acquired hypertriglyceridemia is more or less separate from the common obesity associated elevation of serum triglyceride but it is difficult to segregate between individuals belonging to the two categories we decided to exclude from the present material all individuals with a clearly elevated serum triglyceride level It was

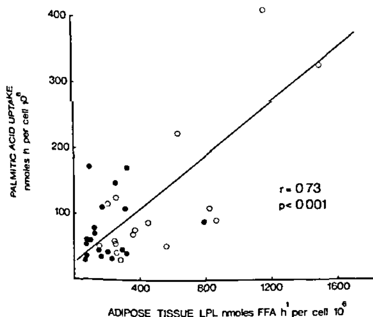


Fig 5 Correlation between fat cell palmitic acid uptake and mean fat cell lipoprotein lipase (*LPL*) activity in obese (O) and non-obese (●) subjects

thought that a comparison of obese and non obese individuals with a normal serum triglyceride level might reveal alterations of triglyceride metabolism caused by obesity itself without admixture of features from a possible primary endogenous hypertriglyceridemia

The frequent occurrence of hypertriglyceridemia in obesity has been related to hyperinsulinemia (8 29 30) and it has been suggested that secretion of increased amounts of insulin accelerates the production of VLDL triglycerides which in turn leads to elevation of serum triglyceride (30) However the relationship between hyperinsulinism and hypertriglyceridemia is not necessarily causal (23) We have previously pointed out that in obese subjects there is hardly any correlation between insulin secretion rate and serum triglyceride concentration (26) The results of the present study support this conclusion further by demonstrating that obese subjects with markedly elevated plasma insulin and exaggerated insulin responses to glucose challenge still had a normal serum triglyceride level It is thus evident that hyperinsulinism or associated over production of VLDL do not suffice to explain the development of hypertriglyceridemia in obesity a failure of removal is also required This view fits best with our present data

In accordance with previous authors (5 6 13 20) we found that the LPL activity of the individual cell was positively correlated to cell size This suggests that the enlarged fat cells of obese people might be capable of utilizing circulating VLDL triglycerides more effectively than cells of normal size This view was in fact confirmed by the present in vitro experiments where the uptake of VLDL triglyceride fatty acids by fat cells of obese people was increased Since the estimated total number of fat cells was similar in obese and non obese subjects it seems justified to conclude that the overall disposal of VLDL triglycerides by adipose tissue was more efficient in obese compared with normal weight subjects Thus if our obese patients produced excessive amounts of VLDL because of their hyperinsulinism they maintained the circulating VLDL mass within the normal range by a more efficient removal in adipose tissue In parallel studies we have shown that the LPL activity of fat cells in obese hypertriglyceridemic patients is lower than in normolipidemic obese subjects but similar to that of non-obese normoglyceridemic subjects (41) We believe therefore that endogenous hypertriglyceridemia develops in obesity when an over production of VLDL is combined with a slight defect in its removal

In spite of the increase in LPL activity in adipose tissue the obese subjects did not show an elevated activity of LPL in postheparin plasma but rather a trend to slightly lower values Accordingly there was no correlation between the LPL activities in adipose tissue and postheparin plasma The reason for this discrepancy is not clear and can only be speculated There are no previous data on correlations of LPL activity in any tissue and in postheparin plasma determined by a selective method

Glad et al (11) have recently claimed that the postheparin plasma lipolytic activity (not LPL) in obesity is inappropriately low in relation to the heparin concentration and that this might indicate some kind of resistance in the release of lipase from tissues into circulation Our present results fit this concept since the obese patients had low postheparin plasma LPL activity in relation to LPL in adipose tissue However in contrast to the above authors we used i v heparin in amounts which give maximal LPL response and therefore it is less likely that a defect in the release mechanism of the enzyme could account for the discrepancy between postheparin plasma and adipose tissue LPL activities

Since the skeletal muscle and myocardium are even more potent sources of LPL than the adipose tissue in animals (7 9 34) and skeletal muscle removes a substantial fraction of exogenous triglycerides in man (37) it is possible that the LPL activity of non adipose tissues is decreased in obese subjects and that this accounts for the inappropriately low postheparin LPL activity Thus in the presence of obesity the removal of circulating triglycerides might be diverted from muscle to adipose tissue and this could form one of the mechanisms making obesity a self perpetuating process

ACKNOWLEDGEMENTS

This study was supported by grants from Nordisk Insulinfond Denmark and the Finnish State Medical Research Council (Academy of Finland) An antiserum was provided by Dr C Ehnholm

REFERENCES

1 Afifi A A & Azen S P Computer orientated approach In Statistical analysis Academic Press New York 1972
2 Albrink M J & Meigs J W Interrelationship be

tween skinfold thickness serum lipids and blood sugar in normal men Am J Clin Nutr 15 255 1964
3 Assimacopoulus Jeannet F Singh A Le Marchand Y Loten E G & Jeanrenaud B Abnormalities in lipogenesis and triglyceride secretion by perfused livers of obesehyperglycaemic (ob/ob) mice Relationship with hyperinsulinaemia Diabetologia 10 155 1974
4 Barter P J & Nestel P J Precursors of plasma triglyceride fatty acids in obesity Metabolism 22 779 1973
5 Bjorntorp P Enzi G Ohlson R Persson B Sponbergs P & Smith U Lipoprotein lipase activity and uptake of exogenous triglycerides in fat cells of different size Horm Metab Res 7 230 1975
6 Campbell R G Faibisoff B & Rodows R G Adipose tissue lipoprotein lipase activity in obesity Diabetes (Suppl) 1 367 1974
7 Enser M Clearing factor lipase in muscle and adipose tissue of pigs Biochem J 136 381 1973
8 Ford W Bozian R C & Knowles H C Interactions of obesity and glucose and insulin levels in hypertriglyceridemia Am J Clin Nutr 21 904 1968
9 de Gasquet P & Péquignot E Lipoprotein lipase activities in adipose tissues heart and diaphragm of the genetically obese mouse (ob/ob) Biochem J 127 445 1972
10 — Adipose tissue lipoprotein lipase activity and cellularity in the genetically obese Zucker rat (fa/fa) Biochem J 132 633 1973
11 Glad B W Wilson D E Flowers C M & Reading J C Abnormal post heparin lipolytic activity in obesity A preliminary note Atherosclerosis 22 491 1975
12 Goldrick R B Havenstein N Carroll K F & Reardon M Effects of overfeeding on lipid and carbohydrate metabolism in lean young adults Metabolism 21 761 1972
13 Guy-Grand B & Bigorie B Effect of fat cell size restrictive diet and diabetes on lipoprotein lipase release by human adipose tissue Horm Metab Res 7 471 1975
14 Harlan W R Oberman A Mitchell R E & Graybiel A Constitutional and environmental factors related to serum lipid and lipoprotein levels Ann Intern Med 66 540 1967
15 Herbert V Lau K S Gottlieb C W & Bleicher S J Coated charcoal immunoassay of insulin J Clin Endocrinol Metab 25 1375 1965
16 Huang T C Chen C P Wefler V & Raftery A A stable reagent for the Liebermann Burchard reaction Application to rapid serum cholesterol determination Anal Chem 33 1405 1961
17 Hume R Prediction of lean body mass from height and weight J Clin Pathol 19 389 1969
18 Huttunen J K Ehnholm C Kekki M & Nikkila E A Post heparin plasma lipoprotein and hepatic lipase in normal subjects and in patients with hypertriglyceridaemia correlations to sex age and various parameters of triglyceride metabolism Clin Sci Mol Med 50 249 1976
19 Huttunen J K Ehnholm C Kinnunen P K & Nikkila E A An immunochemical method for the selective measurement of two triglyceride lipases in human postheparin plasma Clin Chim Acta 63 335 1975
20 Jaillard J Sezille G Fruchart J C Dewailly R & Romon M Etude de l'activité de la lipoprotéine lipase et de la cellularité au niveau du tissu adipeux humain influence de l obésité et du diabète Diabete Metab 2 5 1976
21 Jourdan M Margen S & Bradfield R B The turnover rate of serum glycerides in the lipoproteins of fasting obese women during weight loss Am J Clin Nutr 27 850 1974
22 Kessler G & Lederer H In Automation in analytical chemistry (ed L T Skeggs) p 341 Technicon Symposium 1966
23 Nestel P & Goldrick B Obesity changes in lipid metabolism and the role of insulin Clin Endocrinol Metab 5 313 1976
24 Nestel P J Austin W & Foxman C Lipoprotein lipase content and triglyceride fatty acid uptake in adipose tissue of rats of differing body weights J Lipid Res 10 383 1969
25 Nestel P J & Whyte H M Plasma free fatty acid and triglyceride turnover in obesity Metabolism 17 1122 1968
26 Nikkila E A & Taskinen M R Hypertriglyceridemia and insulin secretion a complex causal relationship In Atherosclerosis Proc of the Second International Symposium (ed R J Jones) pp 220–230 Springer Verlag New York 1970
27 Novak M Colorimetric ultramicromethod for the determination of free fatty acids J Lipid Res 6 431 1965
28 Olefsky J Crapo P A Ginsberg H & Reaven G M Metabolic effects of increased caloric intake in man Metabolism 24 495 1975
29 Olefsky J Reaven G R & Farquhar J W Effects of weight reduction on obesity Studies of lipid and carbohydrate metabolism in normal and hyperlipoproteinemic subjects J Clin Invest 53 64 1974
30 Olefsky J M Farquhar J W & Reaven G M Reappraisal of the role of insulin in hypertriglyceridemia Am J Med 57 551 1974
31 Persson B Lipoprotein lipase activity of human adipose tissue in different types of hyperlipidemia Acta Med Scand 193 447 1973
32 Pykalistö O J Smith P H & Brunzell J D Determinants of human adipose tissue lipoprotein lipase Effect of diabetes and obesity on basal and diet induced activity J Clin Invest 56 1108 1975
33 — Human adipose tissue lipoprotein lipase Comparison of assay methods and expressions of activity Proc Soc Exp Biol Med 148 297 1975
34 Rath E A Hems D A & Beloff-Chain A Lipoprotein lipase activities in tissue of normal and genetically obese (ob/ob) mice Diabetologia 10 261 1974
35 Robertson R P Gavareski D J Henderson J D Porte D J Jr & Bierman E L Accelerated triglyceride secretion A metabolic consequence of obesity J Clin Invest 52 1620 1973

36 Rossner S Backman L & Hallberg D The intravenous fat tolerance test in subjects with massive obesity Acta Med Scand 195 279 1974
37 Rossner S Eklund B Kaijser L Olsson A G & Walldius G Removal of exogenous plasma triglycerides in forearm muscle and subcutaneous tissue of hyper and normotriglyceridaemic men Eur J Clin Invest 6 299 1976
38 Salmon D M W & Hems D A Plasma lipoproteins and the synthesis and turnover of plasma triglyceride in normal and genetically obese mice Biochem J 136 551 1973
39 Sims E A Goldman R F Gluck C M Horton E S Kelleher P C & Rowe D W Experimental obesity in man Trans Assoc Am Physicians 81 153 1968
40 Sjostrom L & Bjorntorp P Body composition and adipose tissue cellularity in human obesity Acta Med Scand 195 201 1974
41 Taskinen M R Nikkila E A & Huttunen J K. Adipose tissue lipoprotein lipase activity in hyperlipidemic subjects relation to postheparin plasma lipoprotein lipase to serum insulin and triglyceride levels Abstract of European Society for Clinical Investigation Eur J Clin Invest In press 1977

Acta Med Scand 202 409–411 1977

A Human Albumin of Placental Origin (Albumin Rhodia) Tested for Tolerance

P Jest

From the Nephrological Department University Hospital Odense Denmark

ABSTRACT Fifty infusions each containing 125 ml 20% human albumin extracted from placental tissue, were tested in clinical practice No effects such as changes in the patient s BP, pulse rate and rectal temperature were noted during the observation period and no side effects such as pain, dyspnoea nasal symptoms exanthema or thrombophlebitis, nor any other symptoms to suggest that the solution was not well tolerated

The growing need for human albumin in clinical medicine has called for new methods for its production The Institut Merieux International Lyon France has produced a solution of human albumin isolated from placental blood extracted from the placental tissue The placental tissue is first fractionated with ethanol using Cohn s alcohol method (5 6) Subsequently the product is purified stabilized sterilized by double filtration and finally heated at 60°C for 10 hours in order to inactivate hepatitis virus B (5 6) The product is characterized by its purity (more than 95% of the protein is albumin) its low salt content (less than 0 65 mEq sodium and less than 0 05 mEq potassium/g of albumin) its stability (5 years at +4 C) and its sterility (free from hepatitis virus B) (3 5 6) The Tissue Typing Laboratory Aarhus Kommunehospital Denmark has examined the solution and found it to be free from HL A antigen (4) The solution is sold in Denmark by Pharma Rhodia under the name of Albumin Rhodia 20%

The purpose of this work has been to test the tolerance of patients to the solution by observing any possible changes in their state of health together with any change in BP pulse rate and rectal temperature as well as the appearance of possible side effects such as pain nasal symptoms dyspnoea exanthema or thrombophlebitis

PATIENTS MATERIAL AND METHODS

Fifty infusions from the same batch were used each containing 125 ml 20% Albumin Rhodia One bottle was discarded because of non sterility The albumin was used in cases of shock hypoalbuminaemia and when starting haemodialysis for priming dialysis filters in patients normally needing priming of the kidneys because of instability of the vascular volume and the ensuing fall in BP

Fifteen patients in the Nephrological Department Odense Hospital were tested Their average age was 58 years Their sex age diagnosis the indication for the present infusion and the number of infusions are given in Table I Several patients received more than one infusion in immediate succession

BP pulse and rectal temperature were measured immediately before ½ hour 1 hour and 1½ hours after starting an infusion In order to allow for variations in BP the mean BP=$D+(S-D)/3$ was used where D=diastolic and S=systolic BP

The duration of the infusion was recorded and a note was made of whether the patient had received blood transfusion or albumin previously Any possible toxic and/or allergic manifestations such as pain dyspnoea nasal symptoms exanthema or thrombophlebitis were recorded on a specially numbered observation sheet

The average values of the patients mean BP pulse rate and rectal temperature were calculated together with the standard deviations (S D) In order to evaluate the changes in these mean values during and after the infusion a bilateral analysis of variance was used (2)

RESULTS

The mean arterial BP was 87 6 mmHg (S D =21 8) before and 83 9 (S D =20 6) after the infusion (Table II) The analysis showed no significant de

Table I *Clinical data on the patients*

Pat no	Sex	Age (y)	Diagnoses	Indication for infusion	No of infusions	Earlier blood transfusions
1	♂	64	Pyelonephritis chr nephrolithiasis	Hypotensio arterialis	4	+
2	♂	61	Pyelonephritis chr	Hypotensio arterialis	2	+
3	♀	51	Glomerulonephritis chr allotransplantatio renis diabetes mellitus	Hypotensio arterialis hypoalbuminaemia	9	+
4	♂	73	Neoplasma vesicae	Hypotensio arterialis	2	+
5	♂	38	Insuff renis non deffinata	Hypotensio arterialis	1	−
6	♀	54	Dibein intoxication lactatacidosis	Priming of artificial kidney	6	?
7	♀	66	Nephritis tubulo-interstititialis acuta	Priming of artificial kidney	4	+
8	♀	62	Ren cysticus	Priming of artificial kidney	3	−
9	♀	41	Cancer ren dxt uraemia	Priming of artificial kidney	1	+
10	♀	56	Hypertensio art malignum cum laesio renis	Priming of artificial kidney	3	+
11	♀	64	Pyelonephritis chr	Priming of artificial kidney	5	+
12	♀	66	Ren cysticus	Priming of artificial kidney	2	−
13	♀	35	Pyelonephritis chr	Priming of artificial kidney	2	+
14	♂	69	Hypertensio art malignum cum laesio renis	Priming of artificial kidney	2 2	+ +
15	♂	65	Nephritis interstitialis e phenacetini	Priming of artificial kidney	3	+

iation in mean BP before and after the infusion, as $F_{95\%}$ (3 125)=2 68>2 15 However there was a broad spread in the distribution of the patients mean BPs An increase in the mean BP was observed after 15 infusions The remaining infusions gave either an unchanged mean BP or a fall One patient s mean BP fell from 73 before infusion to 47 mmHg after This was a patient in haemodialysis who frequently experienced a fall of BP during this procedure The changes in mean BP during infusions of Albumin Rhodia 20% should not be taken as a sign of intolerance to the product

Changes in pulse rate were insignificant in individual patients during infusion The mean pulse rate was 83 0 (S D =20 2) before infusion and 84 0 (S D =18 6) afterwards $F_{95\%}$ (3 125)=2 68>0 285 Like in the mean BP wide deviations were found in pulse rate (Table II) but no significant change was observed as a consequence of the infusion

The average rectal temperature during the infusion of Albumin Rhodia 20% was 37 0°C (S D =

Table II *Mean arterial BP pulse rate and rectal temperature in relation to 44 infusions of Albumin Rhodia 20%*

	At start	Hours after start of infusion		
		½	1	1½
BP (mmHg)				
Average	87 7	85 7	84 0	83 9
S D	21 8	19 9	21 0	[illegible]0 6
$F_{95\%}$ (3 125)=2 68>2 15				
Pulse rate (stroke/min)				
Average	83 0	83 9	84 0	84 0
S D	20 2	21 7	18 7	18 6
$F_{95\%}$ (3 125)=2 68>0 285				
Temperature (°C)				
Average	37 0	36 8	36 8	36 8
S D	1 0	1 1	1 1	[illegible]
$F_{95\%}$ (3 125)=2 68>0 586				

1 0) before the infusion and 36 8 C (S D =1 2) after (Table II) After the 17th 18th 19th and 20th in fusions the temperature fell from 36 6 C to 34 2 C these infusions were all given to the same patient who was in an irreversible state of shock This pa tient s temperatures were measured under condi tions not comparable with the others and were de leted from the statistics $F_{95\%}$ (3 125)=2 68>0 586

Thus no significant change was apparent as a consequence of the infusion In one patient the rec tal temperature increased from 37 9 to 38 5 C This patient was in septic shock and had for some time had a fluctuating temperature In none of the other patients was a rise of temperature observed during infusion

One patient with positive hepatitis B antigen be fore the infusion of Albumin Rhodia 20% continued to have positive hepatitis B antigen None of the other patients became hepatitis B antigen positive Eight of these patients were checked for hepatitis B antigen more than ten months after infusion of Al bumin Rhodia 20%

DISCUSSION

Three previous studies have been published on the tolerance to Albumin Rhodia 20%

Trepo et al (8) infused 136 doses of Albumin Rhodia into 25 patients with hepatic cirrhosis or malnutrition Three cases were noted with raised temperature two with shivering and one with a shock like reaction after the infusion The latter pa tient had received numerous blood transfusions previously and had shown a similar intolerance

Graveleau and Eygonnet (1) studied the effect of and the tolerance to the solution during infusion to premature newborn and undernourished infants A total of 160 doses of 10 or 50 ml were infused into 34 infants In one case a transient exanthema was observed immediately after the second infusion No increase in body temperature or changes in the general condition were observed All other patients showed a good tolerance to the solution

Moynot (7) has studied the clinical and biological effect of the solution in 18 mainly premature new born infants The solution was given with the object of correcting their hypoproteinaemia All patients displayed good tolerance to the solution and an in creased albumin level in the blood Although the albumin level then gradually declined it was still above the original values after 72 hours The serum sodium and serum calcium values remained stable during and after infusion

In the three papers cited the solution turned out to be well tolerated It was given as mentioned above to patients with hepatic cirrhosis or to new born infants I considered it useful to supplement these studies by studying the effect of infusions of Albumin Rhodia 20% on patients of a different kind

In none of the patients examined did the infusions of Albumin Rhodia 20% affect the BP pulse rate or rectal temperature in such a way as to indicate that the solution was not well tolerated It should be noted that the infusion of albumin in priming dial ysis filters takes place during approximately 1 min and even large quantities infused during a short period were well tolerated The contents of electro-lytes and albumin and the requirements of purity of the solution examined did not differ from those of known solutions of non placental origin

REFERENCES

1 Graveleau D & Eygonnet J P Utilisation d une al bumine humaine d origine placentaire en perfusion chez le nourrison dénutri le nouveau né pathologique et le premature Rev Pediatr 1 41 1974
2 Hald A Statistical theory with engineering applica tions Wiley New York 1952
3 Human albumin Institut Merieux International Lyon 1974
4 Kissmeyer F Private information Nov 1975
5 Liataud J Pla J Debrus A Gattel P & Peyron L Preparation de l'albumine humaine à partir de sang hemolyse extrait de placentas congeles I Technique de preparation et qualité du produit 13th International Congress of IABS Budapest 1973 Part A Purifica tions of proteins Dev Biol Stand 27 107 1974
6 — Préparation de l albumine humaine a partir de sang hémolyse extrait de placentas congeles II Elimina tion de certaines impuretes au cours de la purification 13th International Congress of IABS Budapest 1973 Part A Purifications of proteins Dev Biol Stand 27 115 1974
7 Moynot A Perfusion d albumine d origine placentaire chez les nouveau nés atteints d'hypoproteinémie Edi tions medicales et universitaires Paris 1975
8 Trepo Ch Chapny H Sambin P Amat G Bory R Contassot J C & Girard M Interet et tolérance des perfusions i v d albumine d origine placentaire chez les sujets cirrhotiques ou denutris Lyon Med 231 69 1974

Acta Med Scand 202 413–417 1977

Bone Marrow Kinetic Studies on Three Patients with Myelomatosis

Indications for Malignant Proliferation within both the Plasma Cell and Lymphoid Cell Compartments

H Mellstedt D Killander and D Pettersson

From the Department of Medicine Serafimerlasarettet and Radiumhemmet Karolinska Hospital and Institute for Medical Cell Research Medical Nobel Institute Karolinska Institute Stockholm Sweden

ABSTRACT The proliferative activity has been studied in lymphocyte and plasma cell populations of purified bone marrow from three myeloma patients In one of them proliferation was also recorded in blood lymphocytes Immunofluorescence studies using idiotypic antisera against the M-component were performed to identify lymphocytes and plasma cells belonging to the malignant cell clone Pulse incubations with ^{3}H thymidine (^{3}H TdR) were made in vitro Autoradiographic analyses revealed a higher overall ^{3}H TdR labelling index in the myeloma cell populations than in normal control cell populations A few labelled plasma cells were observed in the myeloma cases (2.5–5 %) but the major fraction (11.5–14 %) and intensity of labelled cells were found among the lymphoid cells This indicates that the proliferation of malignant cells in myeloma occurs not only within the plasma cell but also in the lymphoid cell population The results provide additional support for the assumption that B-lymphocytes are part of the malignant cell clone in myelomatosis and most likely precursors to the myeloma plasma cells

In the normal B lymphocyte series there are different stages of cell differentiation from the bone marrow stem cell via the virgin B lymphocyte to the end stage cell represented by the mature immunoglobulin (Ig)-producing plasma cells (10) The corresponding differentiation conditions in human plasma cell myeloma are less well known

An increased number of B lymphocytes has been demonstrated in peripheral blood of untreated patients with plasma cell myeloma The majority of these cells carry surface Ig with the same antigenic (idiotypic) characteristics as the monoclonal Ig found in the patient s serum (1 11 13) The membrane bound idiotypic Ig structures are produced by the lymphocytes themselves (8) These monoclonal lymphocytes are regarded as members of the malignant myeloma cell population This view is supported by the finding that the size of the monoclonal lymphocyte subpopulation is closely correlated to the clinical course of the disease (8 15) These observations support the hypothesis (17) that the target cell for the malignant transformation in myelomatosis may be a B lymphocyte which then gives rise to the whole clone of malignant cells If this assumption holds good one would expect a proliferation of malignant cells within the lymphoid cell compartment combined with a differentiation process towards plasma cells the true myeloma cells The proliferation of malignant cells should therefore not be confined to the plasma cell compartment

In order to investigate these problems we have studied the fraction of DNA synthesizing cells within the lymphocyte and plasma cell populations Bone marrow cells from three myeloma patients and two controls and peripheral blood lymphocytes from one of the myeloma patients were pulse labelled with ^{3}H thymidine (^{3}H TdR) Antiserum against idiotypic Ig structures was used in indirect immunofluorescence (IFL) to stain cells belonging to the malignant cell clone

controls ARG analysis of these lymphocyte sus pensions showed an LI of 15% in patient 3 while only 0 5% were labelled in the controls

DISCUSSION

The proliferating capacity of bone marrow lympho cyte and plasma cell populations was studied in three patients with myeloma using purified bone marrow samples The purity of the cell preparations is comparable with that found by others using the same gradient technique (4 6) The contamination of peripheral blood lymphocytes must be small, as only 8–20% T cells were found, while these donors had 53–75% T lymphocytes in their blood It has been suggested that up to 15% T lymphocytes may be found in the bone marrow parenchyma as a result of recirculating T lymphocytes entering the bone marrow (4 7)

It has been shown earlier (13) that antisera produced against idiotypic determinant on myeloma proteins react only with autologous lymphocytes and plasma cells With our technique plasma cells express these structures only in the cytoplasm but on lymphocytes these characteristics can be detected on the membrane and sometimes in the cytoplasm (15) Hence in myeloma the majority of the bone marrow lymphocytes and plasma cells belong the B cell series and to the same malignant cell clone (8) In the two controls most of the lymphocytic plasmacytic cells belonged to the B cell line i e immature B cells with no detectable Ig cells with surface Ig and cells with cytoplasmic Ig (plasma cells) (4 16 18)

In normal human plasma cells and bone marrow T lymphocytes no incorporation of ^{3}H TdR can be detected after short pulse (2 6) and only B lymphocytes are labelled (4) In peripheral blood he LI is very low (0–1%) under normal conditions (4)

Our ARG analyses showed that the LI values of bone marrow myeloma cell populations as well as the intensity of labelling were higher than in the controls Moreover the LI values of myeloma lymphocytes were greatly increased compared with those of the populations of malignant plasma cells The major part of these lymphocytes carried Ig structures on their cell surface and/or in the cytoplasm that were identical to the serum myeloma protein indicating that cells with different morphology (lymphocytes and plasma cells) belong to the same malignant cell clone Furthermore peripheral blood from one of the patients with a large proportion of monoclonal lymphocytes showed a high LI most likely reflecting a proliferating activity of the monoclonal B cells The results indicate that the malignant proliferation in myeloma takes place not only in plasma cells but also and in particular in malignant monoclonal B lymphoid cells Even if T cells under normal conditions (see above) do not synthesize DNA, one could not completely rule out the possibility that a few normal lymphocytes incorporate ^{3}H TdR as double labelling with IFL and ARG was not performed

The result of the present study supports the assumption that B lymphocytes in myeloma patients having Ig with the same antigenic determinants as the serum myeloma protein might be progenitors of the malignant plasma cells (8 17) as they are under normal benign conditions (10) This interpretation is in line with the observation in a case with chronic lymphocytic leukaemia (CLL) producing monoclonal Ig where the same maturation process has been suggested (5) Thus some of the monoclonal B lymphocytes in CLL identified by idiotypic antiserum differentiate to plasma cells producing the monoclonal serum immunoglobulin

ACKNOWLEDGEMENT

This study was supported by grants from the Swedish Cancer Society and Karolinska Institute Foundation

REFERENCES

1 Abdou N I & Abdou N L The monoclonal nature of lymphocytes in multiple myeloma Ann Intern Med 83 42 1975
2 Bøyum A Separation of lymphocytes and erythrocytes by centrifugation Scand J Clin Lab Invest 21 77 1968
3 Drewinko B Brown B W Humphrey R & Alexanian R Effect of chemotherapy on the labelling index of myeloma cells Cancer 34 526 1974
4 Fauci A Human bone marrow lymphocytes I Distribution of lymphocyte subpopulations in the bone marrow of normal individuals J Clin Invest 56 98 1975
5 Fu S M Winchester R J Feizi T Walzer P & Kunkel H G Idiotypic specificity of surface immunoglobulin and the maturation of leukemia bone-marrow-derived lymphocytes Proc Natl Acad Sci USA 71 4487 1974
6 Geha R S Gatien J G Parkman R Crain J D Rosen F S & Merler E Discontinuous density gradient analysis of human bone marrow Presence of alloantigen responsive PHA unresponsive cells

in normal bone marrow absence of B lymphocytes in the bone marrow of patients with X linked agammaglobulinemia Clin Immunol Immunopathol 2 404 1974

7 Harboe M & Natvig J B Medisinsk immunologi p 23 Universitetsforlaget Oslo 1975

8 Holm G Mellstedt H Pettersson D & Biberfeld P Idiotypic immunoglobulin structures on blood lymphocytes in human plasma cell myeloma Immunol Rev 34 139 1977

9 Holm G Pettersson D Mellstedt H Hedfors E & Bloth B Lymphocyte subpopulation in peripheral blood of healthy donors Characterization by surface markers and lack of selection during purification Clin Exp Immunol 20 443 1975

10 Kreth W H & Herzenberg L A Fluorescence activated cell sorting of human T and B lymphocytes I Direct evidence that lymphocytes with a high density of membrane bound immunoglobulin are precursors to plasmacytes Cell Immunol 12 396 1974

11 Lindstrom F D Hardy W R Eberle B J & Williams R C Jr Multiple myeloma and benign monoclonal gammopathy Differentiation by immunofluorescence of lymphocytes Ann Intern Med 78 837 1973

12 Lundgren G Zukoski C F & Moller G Differential effects of human granulocytes and lymphocytes in human in vitro Clin Exp Immunol 3 817 1968

13 Mellstedt H Hammarstrom S & Holm G Monoclonal lymphocyte population in human plasma cell myeloma Clin Exp Immunol 17 371 1974

14 Mellstedt H & Holm G In vitro studies of lymphocytes from patients with plasma cell myeloma I Stimulation by mitogens and cytotoxic activities Clin Exp Immunol 15 309 1973

15 Mellstedt H Pettersson D & Holm G Monoclonal B lymphocytes in peripheral blood of patients with plasma cell myeloma Relation to activity of the disease Scand J Haematol 16 112 1976

16 Möller G Effect of B-cell mitogens on lymphocyte subpopulations possessing C3 and Fc receptors J Exp Med 139 969 1974

17 Salmon S E & Seligmann M B-cell neoplasia in man Lancet 2 230 1974

18 Vitetta E S & Uhr J W Immunoglobulin-receptors revisited A model for the differentiation of bone marrow-derived lymphocytes is described Science 189 964 1975

Acta Med Scand 202 419–422 1977

Vitamin B_{12} Absorption Determined with a Double Isotope Technique Employing Incomplete Stool Collection

Reliability and Validity in Pernicious Anemia

K Hjelt O Munck E Hippe and O Bärenholdt

From the Departments of Clinical Physiology and Internal Medicine F Glostrup Hospital Glostrup and Department of Internal Medicine C Bispebjerg Hospital Copenhagen Denmark

ABSTRACT In 19 control patients and 10 patients with pernicious anemia (PA), the vitamin B_{12} (B_{12}) absorption was determined simultaneously with whole body counting (FRB_{12}) and with a double isotope technique employing incomplete stool collection (FAB_{12}) The test dose was administered orally and consisted of 8 ml of a 10 ml solution containing 0 5 μg of ^{58}Co-B_{12} (approximately 0 4 μCi) and 2 mg of $^{51}CrCl_3$ (approximately 20 μCi) Two ml of the solution were used as standard In order to follow the passage of the inabsorbable tracer, $^{51}CrCl_3$, the patients were given 25 radiopaque pellets and 4 carmine tablets (2 g) to swallow immediately after the test dose Counting of a 3–4 ml specimen from one of the first two red stools was sufficient for calculating FAB_{12} The findings correlated closely with the FRB_{12} values ($r=0\ 99, N=39, p<0\ 001$) In the control subjects, the FAB_{12} averaged 74% (range 37–88) In the patients with PA given intrinsic factor (IF), the FAB_{12} averaged 40% (range 22–59) When IF was not given, FAB_{12} averaged 2% (range 0–9) The test therefore is a valid indicator of pernicious anemia *Reproducibility* For the mean value of 74% the standard deviation (S D) was 5%, corresponding to a coefficient of variation (CV) of 7% In the patients not given IF, S D was 1% and CV 50%, and in those given IF, S D was 8% and CV 20% This shows the test to be as reliable as whole body counting Carmine tablets proved to be a good indicator of isotope-containing stool The test is easy to perform and requires only a scintillation well-counter No co-operation on the part of the patients is necessary The test is also suitable for outpatients Furthermore it is independent of kidney function and flushing with B_{12} is avoided

The methods commonly used for determining vitamin B_{12} (B_{12}) absorption are the Schilling test the double isotope urinary excretion test (Dicopac®) measurement of the plasma radioactivity the hepatic uptake test and whole body counting Of these tests whole body counting gives the best measure of the absorption of B_{12} (8) but it requires heavy equipment A new double isotope technique employing incomplete stool collection seems very promising (3 4 5 7) However the reports give little information on the reliability i e precision and accuracy, of the test The purpose of this work was to evaluate the test when applied to control subjects and patients with pernicious anemia (PA)

STUDY POPULATION

The study population numbers 10 patients with PA in remission for several months and 19 control subjects The examination started not earlier than 14 days after an injection of B_{12} Neither the controls nor the patients were given parenteral B_{12} during the examination period Neither group included subjects with disorders of the thyroid gland liver diseases leukemia or renal diseases

METHODS

Radiopharmaceuticals

Patients and controls fasted for 12 hours before and 4 hours after administration of ^{58}Co-B_{12} and $^{51}CrCl_3$ The radiopharmaceuticals were given orally in a preparation containing 0 5 μg of B_{12} approximately 0 4 μCi ^{58}Co-B_{12} 2 mg of $CrCl_3$ and approximately 20 μCi $^{51}CrCl_3$ dissolved in

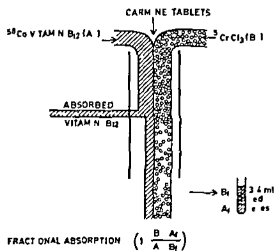


Fig 1 The principle of the incomplete stool collection method for determining the fractional vitamin B_{12} absorption

10 ml of deionized water Two ml of this solution was used as standard The rest plus 25 radiopaque pellets (5) and 4 carmine tablets containing 0 5 g each were given together with 80 ml deionized water Intrinsic factor (IF) was given as hog IF in a dose of 100 mg

Fractional absorption of B_{12} (FAB_{12})

The principle of the test is shown in Fig 1 A sample (3–4 ml) of stool is collected and the amounts of the two in the sample and in the standards are measured a well type sodium iodide crystal connected to a spectrometer using two separate spectrometer windows ^{51}Cr was counted in the energy interval 0 290–0 350 MeV and ^{58}Co in the interval 0 760–0 860 MeV Appropriate correction for the Compton scatter from ^{58}Co was made from counting of standards containing only ^{58}Co-B_{12} in the ^{51}Cr window The stool specimens were not homogenized It is assumed that $^{51}CrCl_3$ is not absorbed from the gut that the two isotopes stay mixed during the passage through the gut that this passage follows that of the intestinal contents and that none of the absorbed ^{58}Co-B_{12} is excreted with the feces during the period of investigation The fractional absorption is calculated from

$$FAB_{12} = 1 - \frac{B_s \times A}{A_s \times B}$$

where B and A are the amounts of $^{51}CrCl_3$ and ^{58}Co-B_{12} respectively given by mouth and B_s and A_s are the respective amounts in the stool specimen

Fractional retention of B_{12} (FRB_{12})

FRB_{12} was measured with a whole body counter as described by Hjelt et al (7 8) at a time when more than 98% of $^{51}CrCl_3$ and all the radiopaque pellets had been excreted (after 7 days in 27 patients after 14 days in 2) In this way the presence of unabsorbed ^{58}Co-B_{12} on the last day of measurement was excluded In the whole body counter

Table I *Percentages for fractional absorption of ^{58}Co B_{12} (FAB_{12}) and for fractional retention of ^{58}Co B_{12} (FRB_{12})*

	FAB_{12}		FRB_{12}	
	Mean	Range	Mean	Range
Control subjects (N=19)	74	37–88	72	39–87
Patients with pernicious anemia (N 10)	2	0–9	5	0–10
Patients with pernicious anemia given IF (N 10)	40	22 59	40	25–65

correction for the Compton scatter from ^{58}Co in the ^{51}Cr window was made by measuring the count rate in the ^{51}Cr window from 3 control subjects and 7 patients with PA given ^{58}Co-B_{12} only

Procedure

The precision was calculated from duplicate determinations The accuracy was assessed by comparing FAB_{12} with FRB_{12} FAB_{12} was determined in three ways 1) Counting of a sample of the first red stool in a well counter 2) Counting of the complete first red stool with the whole body counter 3) Counting of a sample of the second red stool in a well-counter

Counting statistics

The coefficient of variation (CV) for the FRB_{12} values did not exceed 2% The CV for FAB_{12} was maximum 5% when counting was performed with a well counter and 3% when the whole body counter was used

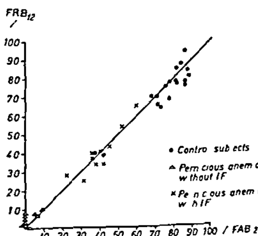


Fig 2 Correlation of the fractional absorption of vitamin B_{12} (FAB_{12}) and the fractional retention of B_{12} (FRB_{12}) in 19 control subjects and in 10 patients with pernicious anemia given and not given intrinsic factor (*IF*)

RESULTS

The first red stool was passed 10–125 hours (mean 40 hours) after administration of the isotopes All the specimens of red stools contained enough radioactivity for the results from counting in a well-counter to be within the above mentioned count rate error

The results are given in Table I In the controls FAB_{12} averaged 74% (range 37–88) and FRB_{12} 72% (range 39–87) In the patients with PA given IF FAB_{12} averaged 40% (range 22–59) and FRB_{12} 40% (range 25–65) When the patients were not given IF FAB_{12} averaged 2% (range 0–9) and FRB_{12} 5% (range 0–10)

The reproducibility was assessed from double assays For the mean value of 74% the standard deviation (S D) was 5% which corresponds to a CV of 7% In the patients not given IF S D was 1% and CV 50% and in those given IF S D was 8% and CV 20% Fig 2 shows a close correlation between FAB_{12} calculated from a sample of the first red stool and FRB_{12} ($r=0\ 99$ $N=39$ $p<0\ 001$) The other two methods of calculating FAB_{12} showed an equally good correlation to the FRB_{12} the coefficients of correlation being 0 99 in both cases ($N=39$ $p<0\ 001$)

DISCUSSION

In his first communication about B_{12} absorption measured with a double isotope technique employing incomplete stool collection Ganatra et al (5) actually used the complete stool In a later short communication (4) it was stated that only an aliquot of the stool sample is needed Without giving any exact data Fish et al (3) stated that 30% of a complete collection is sufficient for diagnostic purposes but only close agreement of the stool and whole body counter methods was attained when greater than 50% of the stool specimen was collected We found close agreement between the two methods when no more than a 3–4 ml specimen from one of the first two red stools was used Counting the complete first red stool in the whole body counter did not change the correlation Therefore a standard scintillation well-counter is sufficient for performing the test

Ganatra et al (5) collected two samples of feces the first 24 hours and the second 48 hours after administration of the test material while Fish et al (3) collected stools from day 1 to day 7 We used carmine tablets for colouring the feces Though counting only a 3–4 ml specimen from one of the first two red stools we obtained an excellent correlation with the results obtained with the whole body counting technique Hinton et al (6) showed that the transit time for radiopaque pellets and powdered carmine correlated well We have shown a good correlation between the passage of non absorbed $^{58}Co\text{-}B_{12}$ and the pellets (8) In agreement with these results our present investigation suggests that the carmine and the isotopes administered have similar transit times In 30% of the B_{12} absorption studies the first red stool arrived more than 72 hours after administration of the test material In such cases stools collected before the first 72 hours probably often contain too little radioactivity for counting! The fact that Ganatra et al (5) had no trouble in collecting samples during the first 48 hours probably reflects a difference between their study population (from India) and our patients (2) with respect to gut transit time We found that the best results were obtained by counting the first red stool but that the next one will give almost identical values

A new absorption study cannot be started until all the non absorbed radioactive material has been excreted As shown by Hjelt et al (8) radiopaque pellets can be used as an indicator of the radioactivity and an X ray of the abdomen therefore will show whether the excretion of pellets is total

The test is excellent in outpatients and it is also very suitable in patients capable of no or minimal co-operation In both these groups the Schilling test is invalidated as it demands complete urine collection (1) Furthermore the method is independent of kidney function and flushing with B_{12} is avoided leaving space for further hematological work up of the patient

REFERENCES

1 Chanarin I & Waters D A W Failed Schilling tests Scand J Haematol 12 245 1974
2 Cummings J H Dietary fibre Gut 14 69 1973
3 Fish M B Pollycove M Wallerstein R O Ka Siu Cheng K & Tono M Simultaneous measurements of free and intrinsic factor (IF) bound vitamin B_{12} (B_{12}) absorption Absolute quantitation with incomplete stool collection and rapid relative measurement using plasma B_{12} (IF) B_{12} absorption ratio J Nucl Med 14 568 1973

4. Ganatra, R. D., Mehan, K. B., Desai, K. B. & Sundaram, K.: Double tracer technique for estimation of vitamin B_{12} absorption. J Nucl Med 12: 357, 1971.
5. Ganatra, R. D., Sundaram, K., Desai, K. B. & Gaitonde, B. B.: Determination of absorption of vitamin B_{12} by a double isotope tracer technique. J Nucl Med 6: 459, 1965.
6. Hinton, J. M., Leonard Jones, J. E. & Young, A. C.: A new method for studying gut transit time using radiopaque markers. Gut 10: 842, 1969.
7. Hjelt, K., Barenholdt, O. & Munck, O.: Determination of fractional vitamin B_{12} absorption with a double isotope technique employing incomplete stool collection. Proc XIII Internat Ann Meeting Soc Nucl Med 78.1–78.4, ISBN 87-7437-493-1, 1975.
8. Hjelt, K., Attrup Rasmussen, P. & Munck, O.: Determination of ^{58}Co-vitamin B_{12} absorption in pernicious anemia by use of whole body counting. Reproducibility and control of gut transit time. Acta Med Scand 201: 167, 1977.

Acta Med Scand 202 423–424 1977

BOOK REVIEWS

The monoclonal gammopathies Multiple myeloma and related plasma-cell disorders By Robert A Kyle and Edwin D Bayrd American Lecture Series 415 pages Charles C Thomas Publisher Springfield Ill USA 1976

This recent monograph by two very experienced clinicians in the field treats a subject that is developing rapidly and therefore needs reviewing It is well known that the connection between the biochemistry of the gamma globulins—or in other words the immunoglobulins—and the clinical picture is very close Almost everything that we know about the structure of these globulins has come from the study of individual patients on the one hand An interesting example of this is the detailed analysis and study of the allergic reagins (IgE) that became possible when it was established that certain myeloma patients are producing large amounts of these proteins in comparatively pure form On the other hand we would not be able to diagnose or treat—possibly abstain from treatment—many of these clinical conditions without intimate knowledge about the physicochemistry immunology or structure of these proteins

This book gives an excellent synopsis of the whole field Modern facts about the chemistry and immunology are treated in a comprehensive way The symptomatology is very clearly defined with stress on the common and important signs even if rare conditions are also mentioned Different opinions regarding the best treatment are discussed extensively and it is clear that the authors have a wide personal experience in this field The fact that the reference list is so extensive is of course very valuable and the number of American authors quoted is impressive Even here there are however some surprising omissions One of the most important facts about the Ig molecule is the division of the Bence Jones proteins into two groups kappa and lambda This was all performed by an American Korngold whose name is not even mentioned European and especially non British contributions are often not quoted even when they have been of fundamental importance but this is of course common in American literature of today

The chapters on macroglobulinemia and amyloid contain excellent information on these subjects and are very valuable The reviewer would regard the special chapters on cryoglobulinemia and pyroglobulinemia as well as on heavy chain diseases as perhaps unnecessarily extensive These interesting phenomena could well have been treated more cursorily in the chapters on myeloma and macroglobulinemia It is very stimulating and of great practical importance that the authors spend several pages on description and discussion of the most common among the gammapathies i e the monoclonal benign This chapter is very valuable and will help many doctors to judge such patients correctly

On the whole it must be said that this is an excellent treatise on a subject that has become very important in oncology The correct diagnosis and treatment of these conditions is described in such a way that both the general internist and the specialist will have much to learn The book is strongly recommended

Jan G Waldenstrom

Thymus and self By Jørgen Rygaard John Wiley and Sons London New York Toronto and Sydney 1973

General interest in thymus pathology has increased recently because of several fundamental discoveries Most spectacular among these is the finding that a special strain of mice are born without a thymus They have no hair and the mutant is therefore called nude In a recent volume Jørgen Rygaard from Denmark who is one of the great authorities on this subject has published his investigations in this field It is impossible to give a synopsis of the many interesting problems that are treated but it is clear that the most fascinating is the fact that these animals do not have any thymus and also have a lack of lymphocytes in the lymph glands The lymphocyte counts in the blood are much lower than in normal mice but it is remarkable that they are not completely alymphocytic as the children with Swiss type hypogammaglobulinemia and lack of thymus The data regarding immunoglobulins in the serum of the nude mouse do not show very remarkable differences from normal On the other hand there must be lack of a thymic humoral factor in these animals

The most remarkable fact is connected with the possibility to graft foreign tissues on these animals Fantastic pictures of skin xenografts are published Human and cat skin may grow for a long time on these animals It has even been possible to put chicken skin with feathers intact that has lived for 32 days Such obvious wonders of nature are of course sensational but they also have a very great biological implication These mice have been used for grafting human tumours These may grow on the mice and their metabolism may be studied in a sort of tissue culture on a living substrate Wortis has studied the occurrence of spontaneous tumours in these mice and interestingly it has been found that they do not develop tumours after painting the skin with a carcinogen Their littermates develop papillomas Most interesting is the fact that nudes develop tumours after a thymus has been grafted on their body

The different ways to explain this include the assumption that the thymus provides a stimulus necessary for tumorigenesis The whole question about the thymus and immunity will be forwarded in a remarkable way by the study of these animals It is a fact however that we need centers specializing in the nursing of these very frail animals in order to keep them alive

The Danish author was the first who realized what a wonderful opportunity these animals offered regarding transplantation of human malignant tumours Already in 1969 he transplanted a highly differentiated adenocarcinoma from the colon and this tumour was passed from nude to nude and was in the summer 1973 in the 32nd passage No distant metastases have been observed The original histological pattern of the tumour is preserved It is clear from Rygaard s work that there is a take of human carcinoma tissue just like any other human tissue may grow On the other hand there is no lowered resistance of the mice as far as metastases are concerned Human leukemia or myeloma does not grow in these animals but the study of effects from anticancer drugs on solid tumours must be ideal Rygaard specially stresses the fact that the experience with a nude mouse seems to invalidate the hypothesis of the immunological surveillance In a total of 10000 nude mice Rygaard has never seen spontaneous malignant disease

The title of the book is Thymus and self These animals must be ideal models for future study of possible humoral factors produced by this organ The book should be read by all doctors and research workers actively interested in immunological and oncological problems

Jan G Waldenstrom

Tumours of the thymus By Juan Rosai and Gerald D Levine 228 pages US $4 95 The Armed Forces Institute of Pathology Washington D C 1975

The excellent series on tumour pathology contains a new volume of great interest both to the clinician and to the anatomical pathologist The conditions of the normal ymus are described briefly but are very well illustrated A chapter on thymic dysplasia in immunodeficiency is teresting and shows that the epithelial cells are well developed with a retained globular configuration There are no Hassals corpuscles and lymphocytes are of course entirely lacking The interesting problem of thymic hyperplasia is also treated but it is remarkable that the fundamental work by Hammar proving that there was a normal involution of the thymus (as early as 1928) is mentioned among the references but is not discussed in the text. Hammar s fundamental and very extensive work put an end to the very popular ideas about status thymicolymphaticus This was for many decades regarded as an indication of a special lymphatic constitution People who were carriers were prone to die from accidents or to become criminals and be executed! The proof was that they all had a big thymus whereas persons who died in hospital from disease had much smaller thymus glands Hammar could show that there was an involution of the thymus occurring in most diseases and also in older age groups The presence of a large thymus in younger people killed in accidents was the normal state!

As always with this series of publications the illustrations are excellent and also the discussion of clinical states connected with thymomas make excellent reading for the clinician It is clear that Castleman who published the previous volume on tumours of the thymus gland in 1955 in the same series is extensively quoted but it is remarkable to find how much new techniques such as electron microscopy has meant for the understanding of thymus pathology

The chapter on symptoms and systemic diseases associated with thymoma is concentrated but contains excellent information Myasthenia gravis is of course the chief complication but also red cell hypoplasia and immunodeficiencies are treated A Scandinavian reader enjoys the fact that Bottiger s remarkable case of pure red cell anemia who recovered after treatment with immunosuppressive drugs is discussed The fact that patients with thymoma may have low serum globulin levels is also interesting The book is a gold mine for everybody interested in problems in this field

Jan G Waldenstrom

Acta Med Scand 202 425–427 1977

Paroxysmal Ventricular Fibrillation in Children

Long term Follow up of Three Cases Treated with β-blocking Agents

Alf Wennevold and Erik Sandøe

From Medical Department B Rigshospitalet (University Hospital) Copenhagen Denmark

ABSTRACT A long term follow up report is given on three children with stress induced bursts of ventricular activity, occasionally proceeding to ventricular fibrillation causing syncope All patients were treated with a β blocking agent as prophylaxis for 12, 10 and 6 years, respectively Case 1 has no signs of organic heart disease She has been followed from the age of 8 years and had her last syncope in 1974 She was last seen in Nov 1976, doing well at the age of 20 Case 2 started having syncopes after an attack of measles at the age of 8 years, at which time she probably acquired some damage to her myocardium She had persistent bradycardia but no other signs of heart disease She had an uneventful pregnancy and delivery in 1973 and gave birth to a normal child She died suddenly in 1974, at the age of 22, four years after her last syncopal attack Case 3 had cardiomyopathy with increasing heart size and exertional dyspnoea and marked ischaemic ECG changes during exercise He was followed from the age of 7 years He died suddenly in 1974 at the age of 16, four years after his last syncope

Cardiac syncopes due to paroxysms of brief malignant tachycardias in children are rare especially when cases with prolonged QT syndrome are excluded (1–6 8) The term brief malignant tachycardia is used for stress induced bursts of ventricular activity occasionally proceeding to ventricular fibrillation causing syncope As the ECG at rest usually shows sinus rhythm the diagnosis has to be established by ECG recording during strenuous exercise or by long term ECG monitoring by means of telemetry or portable tape recorders and the diagnosis should be considered in every child with stress induced syncope

Once the diagnosis has been established the question of long term prophylaxis of these life threatening attacks arises The outcome of such a prophylaxis is still uncertain partly due to the few cases treated and partly to the rather short follow up times published so far

In this paper we report the long term follow up of three children who were treated with β blocking agents because of malignant tachycardia for 12 10 and 6 years respectively The establishment of the diagnosis and the events during the first years of treatment have been published previously (9–13) the emphasis in the case reports below is therefore laid on the later events during the continued treatment

CASE REPORTS

Case 1

A girl born in 1956 with no family history of congenital heart disease She is the second of three children the other two siblings being alive and well Growth and development were normal and she was well until May 1962 when she started having attacks of sudden loss of consciousness in connection with straining She suddenly fell to the ground was pale flaccid and without respiration and she always had incontinence of urine Recovery took place after a few minutes There were no symptoms or complaints in the intervals between the attacks She was first treated for assumed epilepsy without effect

She was seen in our department in 1964 Physical examination chest X ray and resting ECG revealed findings within normal limits ECG after strenuous exercise showed runs of multifocal ventricular extrasystoles thus revealing the true nature of her attacks (10)

Treatment with propranolol was started and the effect was tested on exercise ECGs (13) The dosage of propranolol and the frequency of attacks are shown in Fig 1 She had no attacks for two years but when the treatment was stopped on a test basis she had another attack after 2 weeks and the treatment was resumed She has been maintained on propranolol since and has had only a few attacks usually when she outgrew her dosage or forgot to take the tablets She had her last attack in Nov 1974 She was last seen in Nov 1976 doing well at the age of 20 12 years after initiation of the treatment

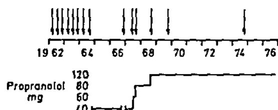


Fig 1 Medical treatment and frequency of syncopes in case 1 Each arrow indicates one syncope

Case 2

An adopted girl born in 1952 without known family history Growth and development were normal and she was well until 1960 when she had measles with high fever and signs of cerebral affection for a few days Three months later she started having syncopes usually in connection with exertion At this time her family doctor noticed persistent bradycardia at rest with 40–48 beats/min

During the following years she was admitted several times to the local hospital following attacks of unconsciousness The attacks inreased in severity lasting up to 40 min often resembling grand mal with convulsions and were now also provoked by emotional disturbances An ECG recorded during the later phase of an attack showed ventricular tachycardia and multifocal ventricular extrasystoles Treatment with quinidine had no effect There were no symptoms between the attacks

She was seen in our department in 1964 Findings at physical examination and chest X ray were normal Resting ECG showed sinus bradycardia but was otherwise normal Right heart catheterization revealed no abnormalities ECG during exercise showed runs of ltifocal ventricular extrasystoles which appeared hin 1 min following the commencement of exercise and rsisted for 4–6 min after exercise These changes were followed by bigeminy for another 6–9 min until sinus rhythm was restored (13)

Various antiarrhythmic drugs were given and the effect was tested on the exercise ECG Propranolol had the best effect as the ectopic activity decreased though it was never completely suppressed (13)

She was then maintained on propranolol The dosage and frequency of attacks are shown in Fig 2 The dosage had to be decreased in the beginning because of drowsiness and bradycardia down to 30 beats/min (12) but could be increased after a few years without inducing side effects Her attacks continued initially though they were less severe From 1970 she had no further attacks (Fig 2)

She had an uneventful abortion in 1970 became pregnant again in 1973 and was delivered by caesarean section in Dec 1973 during uninterrupted treatment with propranolol The pregnancy and delivery were uncomplicated the child is alive and doing well

In Dec 1974 while shopping she suddenly fell down dead on to the floor She was then 22 years old and had been treated with propranolol for 10 years No autopsy was performed Up to the time of her death no change in her cardiac status had been noticed she was feeling well without signs of heart failure

Case 3

A boy born in 1958 with no family history of heart disease He was the first of two children the other sibling being alive and well Growth and development were normal In 1961 at the age of 3 years a heart murmur was discovered At that time he was asymptomatic and an X ray of the chest showed a heart of normal size During the following years it was noted that he tired more easily than his playmates and that he had dyspnoea on exertion From the end of 1964 he started having syncopes on exertion

He was seen in our department in 1965 At auscultation a systolic murmur grade 3 (of 6) was heard at the apex and along the left sternal border The physical examination showed otherwise normal results The chest X ray showed a moderately enlarged heart The resting ECG showed deep Q waves in recordings from lead III and a right bundle branch block pattern ECG during exercise showed marked ischaemic changes which subsided 5 min after completion of the work (11) Right and left heart catheterization showed a slightly elevated end-diastolic pressure in the left ventricle and a small left to-right shunt to the right ventricle only detectable with the hydrogen technique An aortography was misinterpreted as showing a fistula from the left coronary artery to the right ventricle but no fistula was found at thoracotomy The final diagnosis was cardiomyopathy and a small ventricular septal defect

After operation the ECG and heart murmur were unchanged and the patient continued to have syncopes In 1968 we finally succeeded in obtaining an ECG during two

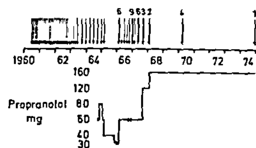


Fig 2 Medical treatment and frequency of syncopes in case 2 The thicker arrows indicate several attacks of syncope within a short time Figures on top of the arrows=no of attacks

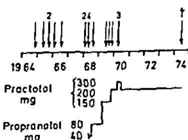


Fig 3 Medical treatment and frequency of syncopes in case 3 Symbols as in Fig 2

of his syncopes by means of continuous long term ECG monitoring partly by telemetry partly by a portable tape recorder Sinus tachycardia was followed by ST depression and ventricular fibrillation which persisted for about 2 min and was followed by asystole lasting about 20 sec when sinus rhythm was restored (9 11)

Treatment with propranolol was started and was later changed to practolol (Fig 3) One of his syncopes had to be treated with a DC counter shock His syncopes disappeared by 1970 He had some feeling of oppression and dyspnoea on exertion but was otherwise doing well In July 1974 he was found dead in the street He died at the age of 16 after 6 years of treatment with β blocking agents No autopsy was performed

DISCUSSION

Our three patients are examples of life threatening syncopes due to paroxysmal ventricular fibrillation of different etiology Case 1 seems to be typical of what has been termed idiopathic recurrent ventricular fibrillation (7) Case 2 developed her first symptoms after an attack of measles at which time she might have acquired some damage to her myocardium Otherwise there were no signs of heart disease so she may belong to the same group as case 1 Case 3 no doubt had cardiomyopathy with increasing heart size and dyspnoea on exertion

Regarding the prophylactic treatment we chose a β blocking agent in all three patients In cases 1 and 2 the effect was tested on the exercise ECG (13) and in case 2 several other antiarrhythmic drugs were tried but propranolol proved to be best After some years we succeeded in abolishing the fainting spells in all patients and observed no serious side effects Case 2 underwent pregnancy and delivery during continuous treatment with propranolol and gave birth to a normal child

It was to be expected that the prognosis would be poor in case 3 with cardiomyopathy but his sudden death after 4 years without syncopes still was surprising So was the sudden death in case 2 after 4 years without syncopes In retrospect we must admit that the prophylactic treatment with a β-blocking agent was successful in only one of our three patients (case 1) Most likely a better result might have been achieved in case 2 with bradycardia if the antiarrhythmic drug had been combined with pacemaker implantation (3)

Our remaining patient case 1 is doing well She experienced her last syncope two years ago Considering that she has no organic heart disease it is to be hoped that her prognosis will be better than that of the others

REFERENCES

1 Akaishi K & Yokoyama T Two cases of Adams Stokes syndrome—with special reference to electrocardiograms in attack Ann Paediatr Jap 8 244 1962
2 Berg K J Multifocal ventricular extrasystoles with Adams Stokes syndrome in siblings Am Heart J 60 965 1960
3 Cohen L S Buccino R A Morrow A G & Braunwald E Recurrent ventricular tachycardia and fibrillation treated with a combination of beta adrenergic blockade and electrical pacing Ann Intern Med 66 945 1967
4 Hernandez A Strauss A Kleiger R E & Goldring D Idiopathic paroxysmal ventricular tachycardia in infants and children J Pediatr 86 182 1975
5 Horan M & Venables A W Paroxysmal tachycardia with episodic unconsciousness Arch Dis Child 37 82 1962
6 Kast G Paroxysmales Kammerflattern mit Adams-Stokesschen Anfallen bei einem 15 jahrigen Madchen Z Kreislaufforsch 57 256 1968
7 Ledwich J R & Fay J E Idiopathic recurrent ventricular fibrillation Am J Cardiol 24 255 1969
8 Lenartowska I & Swiderski J Paroxysmal ventricular arrhythmia seizures and electrolyte disturbance syndrome Pol Med Sci Hist Bull 6 68 1963
9 Videbæk J Andersen E D Jacobsen J R Sandøe E & Wennevold A Paroxysmal tachycardia in infancy and childhood II Paroxysmal ventricular tachycardia and fibrillation Acta Paediatr Scand 62 349 1973
10 Wennevold A Melchior J C & Sandøe E Adams Stokes syndrome in childhood without organic heart disease Electrocardiogram after exercise as a diagnostic tool Acta Med Scand 177 557 1965
11 Wennevold A & Sandøe E Paroxysmal malignant arrhythmias in childhood and adolescence In Symposium on Cardiac Arrhythmias Elsinore Denmark 1970 (ed E Sandøe E Flensted Jensen & K H Olesen) p 767 Astra Sodertalje 1970
12 — Propranolol (Inderal) in the long term prophylaxis of ventricular arrhythmias Acta Med Scand 183 87 1968
13 Wennevold A Sandøe E & Melchior J C Propranolol (Inderal) in the management of Adams Stokes syndrome in childhood Acta Med Scand 178 483 1965

Acta Med Scand 202 429–431 1977

Cardiomyopathy after Chloroquine Treatment

I Magnussen and B de Fine Olivarius

From the University Department of Neurology Århus Kommunehospital Århus Denmark

ABSTRACT A case of cardiomyopathy combined with vacuolar myopathy in the extremity muscles and irreversible retinopathy produced by chloroquine is described

Chloroquine has been known as one of the more effective and less toxic of the quinoline derivatives introduced during and after World War II as antimalarials It has also been advocated in the long term treatment of certain skin diseases scleroderma lupus erythematosus discoides pemphigus and lichen planus as well as in some generalized disorders as rheumatoid arthritis LED and polymyositis

Among the toxic side effects of the drug attention has mainly focussed on the eye changes with reversible corneal and irreversible retinal lesions with macular degeneration (9)

Another rare but well documented complication to long term treatment with chloroquine or its congeners is a neuromyopathy with proximal weakness and wasting of extremity muscles associated with diminished tendon reflexes (2–12 16 18–20 22 24 29) The pathological changes in voluntary muscle have been described as a vacuolar myopathy (7–12 16–19 22 24) Clinically the neuromuscular lesions are reversible in most cases after discontinuation of the drug

In experimental studies with chloroquine given to rats for up to two years weakness of voluntary muscles was observed the main histological change being a vacuolar myopathy (21) and similar though still more pronounced histological changes were seen in the myocardium with preference for the interventricular septum in rabbits (27)

In man similar histological changes in the cardiac muscle have been described incidentally in only one patient who died after two years of treatment with chloroquine and who presented clinically with both a neuromyopathy and cardiac failure (17)

Furthermore EEG changes have been commented on only occasionally (12 24 29) usually as a bundle branch block which was reversible in the case of Whisnant et al (29)

Apart from this cardiomyopathy does not seem to have been referred to in the literature as a specific complication to chloroquine treatment and we therefore find it of some interest to report on the first fully investigated patient with cardiomyopathy subjected to a long term follow up

CASE REPORT

The patient a 35 year old woman had suffered since childhood from a rather severe psoriasis For seven years she had been treated continuously for this disorder with chloroquine phosphate (Resochin®) in doses of 250 mg four times a day—a total dose of 2 5 kg of chloroquine Otherwise she had previously been healthy especially without symptoms or signs of cardiovascular disease No other drugs had been given especially no corticosteroids

For one year prior to admission she had complained of dimness of vision and a weight loss of 4 kg Her hair had turned definitely more blonde and she had developed redness of the skin on the face as well as the fingers She had noticed a tight feeling in the hamstrings and a progressive weakness of the legs with difficulty in walking especially in climbing stairs During the same period she had increasing dyspnoea on exertion and a tendency to ankle oedemas Her symptoms had subsided somewhat after discontinuation of chloroquine one month before admission

She was first admitted in May 1971 Clinical examination showed that her facial complexion was remarkably red and so was the skin of the fingers while her hair was very fair There were moderately pronounced dispersed elements typical of psoriasis in the skin Slight wasting of the shoulder girdle and of the thenar muscles was noted and slight weakness of the extension of both elbows The biceps reflexes were weak the other tendon reflexes absent In the lower extremities there was a moderate

symmetrical wasting of both quadriceps muscles and bilateral foot drop There were severe pareses of the flexion of both hips and of the dorsiflexion of both feet and all toes All tendon reflexes were absent in the legs plantar responses normal No sensory disturbances were found The gait was paretic

Special investigations

Electromyography (both anterior tibial muscles) showed shortened potential duration low amplitude of potentials and polyphasia as signs of a myogenic affection Motor and sensory nerve conductions were normal

Muscle biopsy (left gastrocnemius) showed the picture of a severe necrotising vacuolar myopathy compatible with chloroquine intoxication

ECG showed regular sinus rhythm (88/mm) incomplete RBBB left ventricular hypertrophy and left ventricular strain

Cardiological examination (including heart catheterization) showed slightly elevated pressure in the right atrium X ray of the chest (including contrast in oesophagus) was normal

Ophthalmological examination revealed normal visual acuity Ophthalmoscopic examination showed very narrow retinal vessels and bilateral central retinal pigmentation and large ring scotomas were found in both visual fields

BP was 130/70 Hb WBC serum aldolase serum LDH CPK electrolytes and all relevant tests for collagen or endocrine disorders were normal

The patient was treated with physiotherapy and during the following 5 months she improved considerably The gait was nearly normalized although the power in the feet was still a little weak She gained 6 kg in weight during that time There was now no wasting or weakness of the upper xtremities but tendon reflexes were still weak There as slight bilateral wasting of the quadriceps femoris but no pareses over the hips or knees still moderate symmetrical weakness of the dorsiflexion of the feet and toes The patellar reflexes were normal the achilles tendon reflexes were still absent No sensory disturbances Gait normal She had no cardiac complaints

In Feb 1976 the patient was readmitted for a full neurological and cardiological examination after 5 years off the drug She had still some weakness of the feet but no symptoms of cardiac incompensation The skin of the face was still somewhat reddish and she had accentuated psoriatic elements of the skin Some dimness of vision still persisted Apart from diminished tendon reflexes the upper extremities were normal Bilateral wasting of both quadriceps muscles and slight pareses over the hips were still observed as well as a moderate symmetrical weakness of the dorsiflexion of both feet and toes She had no sensory disturbances and the gait was normal

Special investigations

Electromyography (left m vastus medialis) still showed definite signs of a myogenic affection

Muscle biopsy (m triceps surae) showed unspecific degenerative changes without vacuoles but many fibres did show the picture of splitting and were coloured intensely eosinophilic

ECG showed unchanged RBBB and signs of myocardial damage

DISCUSSION

Our patient presented with a neuromyopathy with changes in voluntary muscles typical of chloroquine intoxication and with visual symptoms and typical retinal changes after 7 years of treatment with a large total dose of chloroquine for a life long psoriasis Considering previous reports on such complications to chloroquine intoxication and the partial regression of the neuromuscular symptoms and signs after discontinuation of the drug we consider it beyond any doubt that the complications were due to the excessive and prolonged intake of chloroquine

Vacuolar myopathy of skeletal muscle has also been described in a number of other conditions systemic lupus hypokalaemic periodic paralysis dermatomyositis polymyositis and thyrotoxic myopathy as well as secondary to steroid therapy In our patient there was no evidence of collagen or endocrine disease and no derangement of electrolytes Furthermore no other drugs had been given

Psoriasis is not known to induce vacuolar degeneration of muscles Neither can psoriasis produce the typical retinal changes and visual field defects seen after long term treatment with chloroquine (9)

As the cardiac symptoms and signs developed concomitantly with the other complications we feel confident that the cardiac disease was also due to the chloroquine medication Although chloroquine in animal experiments produces even more pronounced and constant changes in the myocardium than in skeletal muscles (27) only one patient has been published with myocardial damage after prolonged administration of chloroquine (17) Rewcastle and Humphrey (24) describe a patient with neuromyopathy corneal and ECG changes consisting of left axis deviation and RBBB but without cardiac symptoms and signs In our case initial subjective and clinical symptoms of cardiac decompensation completely subsided after discontinuation of the drug but persistent ECG conduction disturbances were st[illegible] ent 5 years after the treatment

Diminution of T [illegible] ne or [illegible] the ECG or interventri[illegible] ay b[illegible] y ch[illegible] , 2 [illegible] et al [illegible] t

all changes in the ECG were reversible when chloroquine was administered for no more than 77 days The ECG changes may certainly be due to an effect of chloroquine on the excitable tissues (15 28) Chloroquine has even been advocated as drug therapy for rhythm disturbances of the heart (13)

As to the pathogenesis electronmicroscopic studies have indicated that chloroquine attacks mitochondria and the more pronounced affection of heart muscle than skeletal muscle in experimental studies fits well with the fact that heart muscle is more rich in mitochondria than are the granular fibres of skeletal muscle In this connection the very rare reports on cardiac disorder in chloroquine intoxication can be explained by the lack of specific attention to and examination for cardiac complications in most cases described in the literature

Cardiomyopathy has been widely discussed in recent years (14) the condition being described in primary (idiopathic) and secondary forms with a wide spectrum of etiological possibilities We think that our case can be labelled as a secondary non familiar congestive toxic cardiomyopathy and that chloroquine should be added to the etiological possibilities in otherwise obscure organic cardiac diseases without hypertension and atherosclerosis It also seems relevant to warn against the injudicious use of chloroquine for trivial disorders and to recommend regular controls including the heart condition in patients on long term treatment for disorders in which chloroquine really is therapeutically indicated

REFERENCES

1 Alving A S Eichelberger L Craige B Jr Jones R Whorton C M & Pullman T N Studies on the chronic toxicity of chloroquine (SN–7618) J Clin Invest 27 60 1948
2 Arnold H L Dermatological pharmaceutics Straub Clin Proc 28 130 1962
3 Begg T B & Simpson J A Chloroquine neuromyopathy Br Med J 1 770 1964
4 Blom S & Lundberg P O Reversible myopathy in chloroquine treatment Acta Med Scand 177 685 1965
5 Bonard E C Neuropathie durch Chloroquin Medikation Schweiz Med Wochenschr 96 1103 1966
6 Dubois E L Systemic lupus erythematosus Recent advances in its diagnosis and treatment Ann Intern Med 45 163 1956
7 Eadie M J & Ferrier T M Chloroquine myopathy J Neurol Neurosurg Psychiatry 29 331 1966
8 Ebringer A Chloroquine myopathy Br Med J 2 770 1971
9 Ebringer A & Colville P Chloroquine neuromyopathy associated with keratopathy and retinopathy Br Med J 2 219 1967
10 Garcin R Rondot P & Fardeau M Sur les accidents neuromusculaires et en particulier sur une myopathie vacuolaire observes au d un traitement prolonge par la chloroquine Rev Neurol 111 177 1964
11 Gerard J M Stoupel N Collier A & Flament Durand J Morphologic study of a neuromyopathy caused by prolonged chloroquine treatment Eur Neurol 9 363 1973
12 Girard P L Dumas M Sarrat Y Dotton B & Heraut L Neuropathie a la chloroquine Bull Soc Med Afr Noire Lang Fr 18 176 1973
13 Goodman L S & Gilman A The pharmacological basis of therapeutics p 1050 Macmillan New York 1975
14 Hartel G Kardiomyopatier Medicinsk Årbog Munksgaard Copenhagen 1975
15 Hess M E & Schmidt C F Cardiovascular effects of chloroquine with special reference to its antifibrillatory action Circ Res VII 86 1958
16 Hicklin J A Chloroquine neuromyopathy Ann Phys Med 9 189 1968
17 Hughes J T Esiri M Oxbury J M & Whitty C W M Chloroquine myopathy Q J Med 11 85 1971
18 Itabashi H H & Kokmen E Chloroquine neuromyopathy Arch Path 93 209 1972
19 Lang P A Smith G H & Green W O Vacuolar myopathy in lupus erythematosus JAMA 191 49 1965
20 Loftus L R Peripheral neuropathy following chloroquine therapy Can Med Assoc J 89 917 1963
21 Nelson A A & Fitzhugh O G Chloroquine (SN–7618) Arch Path 45 454 1948
22 Olivarius B de Fine & Dalby M Chloroquine neuromyopathy (abstr) Excerpta Med 334 176 1974
23 Read W K & Bay W W Basic cellular lesion in chloroquine toxicity Lab Invest 24 246 1971
24 Rewcastle N B & Humphrey J G Vacuolar myopathy Arch Neurol 12 570 1965
25 Rubin M Bernstein H N & Zvaifler N J Studies on the pharmacology of chloroquine Arch Ophthalmol 70 474 1963
26 Sangvhi L M & Mathur B B Electrocardiogram after chloroquine and emetine Circulation 32 281 1965
27 Smith B & O Grady F Experimental chloroquine myopathy J Neurol Neurosurg Psychiatry 29 255 1966
28 Vartanian G A & Chinyanga H M The mechanism of acute neuromuscular weakness induced by chloroquine Can J Physiol Pharmacol 50 1099 1972
29 Whisnant J P Espinosa R E Kierland R R & Lambert E H Chloroquine neuromyopathy Proc Mayo Clin 38 501 1963

The 8th Meeting of the European Poison Control Centres and *the Annual European Meeting of the International Association of Forensic Toxicologists* will be held in Utrecht the Netherlands July 4–7 1978 in cooperation with the American Association of Poison Control Centers and the American Academy of Clinical Toxicology The official language of the meetings will be English

Organization Committee Professor Dr A N P van Heijst and Professor Dr R A A Maes

Secretariat 8th Meeting of the European Poison Control Centres and European Meeting of TIAFT Centre for Human Toxicology State University Vondellaan 14 Utrecht The Netherlands

Current and impending technology for therapy and toxicological analyses as these can be applied to the diagnosis and treatment of poisoned patients will be discussed and explored Plenary sessions will be keynoted by distinguished contributors and will be followed by invited and preferred papers from the participants Papers may be submitted by any registered participant The final date for receipt of abstracts is Jan 31 1978

The Second World Congress on Pain will be held at the Queen Elizabeth Hotel in Montreal Canada Aug 27–Sept 1 1978 Official language of the Congress will be English

Plenary session themes Peripheral nerve lesions and pain Low back pain Orofacial pain and headache Endogenous opiates and pain control Pain measurement in man and animals Pain control by afferent stimulation

Enquiries Secretariat Second World Congress on Pain 3587 University Street Montreal Quebec H3A 2B1 Canada

Acta Med Scand 202 449–454 1977

Prostaglandin-Induced Diarrhoea Treated with Loperamide or Diphenoxylate

A Double Blind Study

Aksel P Lange Niels J Secher and Willem Amery

*From the Department of Gynaecology and Obstetrics
Odense Sygehus Odense Denmark*

ABSTRACT Loperamide was compared double blind with diphenoxylate and a placebo in 59 women with diarrhoea due to prostaglandin administration for mid trimester abortion Treatment was started with the intake of two capsules two hours before the first intramuscular injection of 15(S)15 methyl prostaglandin $F_2\alpha$ and was then adapted individually, i e one capsule after each unformed stool, with a maximum of ten per 24 hours Both antidiarrhoeals were significantly more effective than the placebo in preventing diarrhoea, and loperamide was found to be more active than diphenoxylate The course of abortion, BP and vital signs, or prostaglandin side effects other than diarrhoea were not affected by either antidiarrhoeal, nor could any adverse experience be specifically attributed to them

The synthesis of 15(S)15 methyl prostaglandin $F_2\alpha$ (15 methyl $PGF_2\alpha$) has provided us with a substance which is more slowly catabolized in the body than $PGF_2\alpha$ itself and which therefore is more and especially longer active However the side-effects with $PGF_2\alpha$ are also caused by this derivative and nausea vomiting and diarrhoea are thus common to the 15(S)15 methyl $PGF_2\alpha$ treatment As the latter substance seems particularly useful in inducing late abortions (21) and labour (28) the availability of drugs which are able to antagonize these side-effects and which carry no appreciable risk of side effects of their own should be a very welcome tool in optimizing prostaglandin treatment for inducing abortion

Diphenoxylate has been successfully used for years in the symptomatic treatment of diarrhoea (4 14 22) and is considered a reference substance for similar new drugs Such a new drug is loperamide Extensive pharmacological studies (2 16 17 18 19 27) have demonstrated that loperamide is a potent and orally active drug with a very long duration of action that it is considerably more active than diphenoxylate and that it probably becomes tightly bound to the nerve structures in the intestinal wall also this substance has been reported to have a wide safety margin (13 16) and to be devoid of morphine like central effects (3 8 17) The clinical effectiveness of loperamide has already been demonstrated in clinical trials on both acute (1 15 20) and chronic (5 7 12 23 24 25) diarrhoea and so has its superiority to diphenoxylate in acute (1 6) and chronic (19 26) cases The efficacy of diphenoxylate in preventing prostaglandin induced diarrhoea has already been reported (9 11) When we started this study no data were available about the possible effect of loperamide on prostaglandin diarrhoea but in the meantime it has been shown that this new substance also antagonizes the effects of prostaglandins on the gut (10)

We felt that loperamide deserved a close evaluation in 15-methyl $PGF_2\alpha$ induced diarrhoea Our experience is reported in this paper

STUDY POPULATION AND METHODS

Patients

Fifty nine women admitted to the hospital for mid trimester abortion were studied Only asthma and serious heart or lung diseases were adopted as criteria for exclusion

The age weight and height of the patients are presented in Table I

Table I *Patient data*
Median values (range within parentheses)

	Loperamide group (*N*=24)	*Diphenoxylate* group (*N*=25)	*Placebo* group (*N*=10)	*Total* population (*N*=59)
Age (y)	19 (15–42)	23 (15–40)	20 (15–37)	21 (15–42)
Weight (kg)	51 (37–81)	59 (47–98)	53 (42–128)	54 (37–128)
Height (m)	1 63 (1 46–1 73)	1 62 (1 43–1 76)	1 64 (1 60–1 67)	1 63 (1 43–1 76)

Experimental design and medication

Prostaglandin treatment Two millilitre ampoules containing 1 mg 15(S)15 methyl $PGF_2\alpha$ (500 μg/ml) and stored in the refrigerator were put at our disposal for induction of abortion The drug was injected i m every second hour (250 μg i e 0 5 ml/injection) until abortion was completed however, changes of the dose and/or the interval were allowed if the effect of this treatment was considered insufficient or exaggerated (as judged from the occurrence of violent abdominal pains and the presence of a very hard hypertonic uterus)

Double blind antidiarrhoeal treatment The antidiarrhoeal drug supply was strictly double blind and consisted of two series of 30 packages each containing 20 capsules (one package of the second series could not be used however because of faulty manipulation) The use of a placebo was limited to the second part of the trial (i e second series of packages) whilst the two active preparations (Fig 1) were used throughout the study The composition of the packages was therefore as follows First series 15 packages of loperamide (2 mg/capsule) 15 packages of diphenoxylate (2 5 mg/capsule) Second series 9 packages of loperamide (2 mg/capsule) 10 packages of diphenoxylate (2 5 mg/capsule) 10 packages of a placebo The packages were individually numbered according to the chronological trial numbers of the patients The composition of each package was determined by randomization

This double blind medication was used as follows The treatment started with the intake of two capsules two hours before the first 15 methyl $PGF_2\alpha$ administration thereafter the treatment was individually adapted as each patient was requested to take one capsule (from the same package bearing her number) after each unformed stool A maximum dose of ten capsules per 24 hours was possible

The 15 methyl $PGF_2\alpha$ solution was put at our disposal by The Upjohn Co Kalamazoo Mich USA diphenoxylate (Retardin® Lomotil®) loperamide (Imodium®) and the double blind supply of placebo by Janssen Pharmaceutica Beerse Belgium

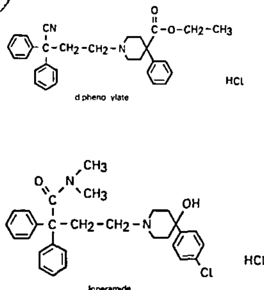


Fig 1 Structural formulae of loperamide and diphenoxylate

Other treatments *Prochlorperazine* was used to prevent nausea and vomiting For that purpose 1 ml of the commercially available solution containing 12 5 mg/ml was injected i m half an hour before initiation of the prostaglandin treatment These injections were repeated every 4–6 hours until abortion had occurred *Pethidine* was preferred as a major analgesic because it has only marginal effects if any on the gut *Other drugs* especially those with possible antidiarrhoeal potential were explicitly prohibited

Other measures The patients were not confined to their beds and were allowed to go to the lavatory

Assessments

The following data were carefully registered for each patient Medication used dose and hour of administration or intake Time of occurrence of prostaglandin side-effects (vomiting episodes and unformed stools) Time course of the effects of the prostaglandin treatment on the uterus using the following criteria first occurrence of labour pain first vaginal blood loss and possible discharge of water occurrence of abortion these data were completed by registering the time of the curettage Monitoring every three hours of systolic and diastolic BP heart rate respiratory rate and body temperature Possible side-effects of the double blind treatment and time of their occurrence

Table II *Time course of the abortion (hours) after 15 methyl $PGF_2\alpha$*
Median values (range within parentheses)

	Loperamide group	Diphenoxylate group	Placebo group	Total population
First labour pain	2 (0–19 10)	2 23 (0–12)	4 23 (0 30–22 30)	2 15 (0–22 30)
Start of bleeding	7 (1–28)	7 55 (2 30–23)	10 15 (0.30–34 30)	7 (0 30–34 30)
Discharge of water (if any)	13 15 (4–19 10)	11 (2 45–26 15)	16 25 (12 30–21 15)	12 30 (2 45–26 15)
Occurrence of abortion	14 30 (7–36 45)	11 (3 20–26 15)	16 08 (6 30–40 45)	15 35 (3 20–40 45)
Curettage	16 (9 30–36 45)	19 (8–26 15)	33 38 (24 30–42 45)	19 (8–42 45)

Statistical evaluations

The comparability of the groups was assessed by means of the two-tailed median test to check whether the randomization procedure had been successful

The same test was used to evaluate the effect of the double blind medication on Possible side-effects other than diarrhoea of prostaglandin treatment (two-tailed probability) The occurrence of diarrhoea provoked by the abortion inducing treatment (one tailed probability) As the intake of double blind medication had been shaped to the occurrence of diarrhoea the total amount of this medication used was an indication of the individual need of an antidiarrhoeal drug This figure was divided by the total amount of 15-methyl $PGF_2\alpha$ used in order to correct interindividual differences in the total dose used of this diarrhoea inducing treatment

For comparisons with the placebo were considered only patients who had been randomized for that purpose (i e those who had used medication from the second series of 30 double blind packages) For comparisons between loperamide and diphenoxylate were considered all patients who had used these drugs

Table III *Evolution of BP heart rate respiratory rate and temperature*
Mean (±S E)

	Hours after medication				
	0	3	6	9	12
Systolic BP (mmHg)					
Loperamide	122 4 (±2 8)	120 2 (±3 0)	121 2 (±2 5)	126 5 (±3 7)	131 5 (±4 4)
Diphenoxylate	117 7 (±2 3)	116 7 (±2 3)	121 3 (±3 1)	121 8 (±2 2)	121 9 (±3 4)
Placebo	126 0 (±6 2)	127 0 (±8 8)	120 6 (±3 6)	127 9 (±4 6)	141 4 (±18 3)
Diastolic BP (mmHg)					
Loperamide	74 8 (±2 8)	72 9 (±2 5)	72 0 (±1 5)	73 0 (±1 9)	74 6 (±2 9)
Diphenoxylate	71 3 (±3 0)	72 7 (±2 4)	72 5 (±2 8)	67 9 (±2 1)	68 6 (±3 0)
Placebo	72 5 (±5 8)	72 0 (±4 8)	70 0 (±3 1)	65 8 (±7 1)	74 2 (±11 7)
Heart rate (beats/min)					
Loperamide	80 4 (±2 8)	77 2 (±3 0)	78 6 (±2 4)	82 7 (±2 5)	90 7 (±3 1)
Diphenoxylate	77 2 (±2 8)	76 4 (±3 5)	82 3 (±3 7)	85 7 (±3 0)	89 1 (±4 1)
Placebo	80 8 (±3 3)	76 0 (±4 1)	85 5 (±4 9)	88 6 (±4 5)	89 6 (±4 9)
Respiratory rate/min					
Loperamide	20 4 (±0 8)	21 3 (±1 1)	20 3 (±0 7)	21 1 (±0 8)	20 1 (±0 8)
Diphenoxylate	20 4 (±0 9)	20 7 (±0 8)	19 3 (±0 9)	19 9 (±0 7)	20 3 (±1 2)
Placebo	19 3 (±1 2)	19 8 (±1 2)	19 7 (±1 3)	18 6 (±1 1)	18 2 (±1 9)
Body temperature (°C)					
Loperamide	36 9 (±0 07)	36 6 (±0 1)	36 9 (±0 1)	37 2 (±0 08)	37 3 (±0 09)
Diphenoxylate	36 7 (±0 08)	36 5 (±0 09)	37 0 (±0 09)	37 4 (±0 07)	37 3 (±0 1)
Placebo	36 9 (±0 1)	36 6 (±0 1)	37 0 (±0 2)	37 1 (±0 2)	37 2 (±0 1)

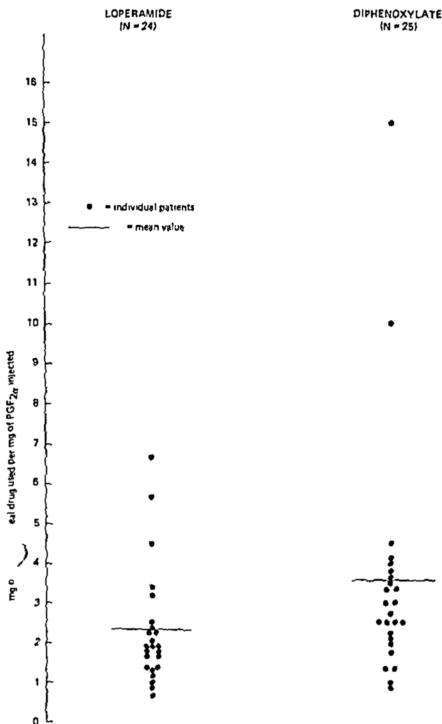


Fig 2 Relative need of antidiarrhoeal drugs

RESULTS

As stated above 59 patients were evaluable breaking of the code revealed that the three treatment groups (24 patients on loperamide 25 on diphenoxylate and 10 on placebo) were well comparable regarding age, weight and height

The time course of abortion phenomena is summarized in Table II No significant differences were observed between either treatment group

No significant differences were found regarding BP, respiratory rate, body temperature heart rate (Table III) use of pethidine and occurrence of vomiting

The number of double blind capsules taken per 1 ml of injected 15 methyl $PGF_2\alpha$ solution was significantly higher with the placebo than with loperamide ($p = 0.05$) but the difference with diphenoxylate fell just short of statistical significance ($p = 0.08$) The mean number of capsules taken per 2 ml of the 15 methyl $PGF_2\alpha$ solution was 1 3 with the placebo 0 79 with loperamide and 0 89 with diphenoxylate Also the amount of drug (mg) used per 2 ml of the prostaglandin solution was significantly higher with diphenoxylate than with loperamide ($p = 0.008$) The mean amounts were 3 5 mg with diphenoxylate as compared with 2 3 mg with loperamide suggesting that the latter is at least 1 5 times more active than the reference substance in this type of diarrhoea (Fig 2)

No side-effects related to the use of the antidiarrhoeal drugs were observed or reported

DISCUSSION

Besides confirming the usefulness of prostaglandins in inducing mid trimester abortion this study has provided evidence that loperamide and possibly also diphenoxylate may prevent prostaglandin induced diarrhoea without affecting the course of the abortion or the other side effects of prostaglandin Also on a weight basis loperamide was found to be more active than diphenoxylate

As the loperamide treatment appears almost free from side-effects and is *more active than diphenoxylate* its use in preventing prostaglandin induced diarrhoea is recommendable

REFERENCES

1 Amery W Duyck F Polak J & van den Bouwhuysen. G A multicentre double blind study in acute diarrhoea comparing loperamide with two common antidiarrhoeal agents and a placebo Curr Ther Res 17 263 1975
2 Awouters F Niemegeers C J E Kuyps J & Janssen P A J Loperamide antagonism of castor oil-induced diarrhea in rats a quantitative study Arch Int Pharmacodyn Ther 217 29 1975
3 Colpaert F C Niemegeers C J E Lal H & Janssen P A J Investigations on drug produced and subjectively experienced discriminative stimuli 2 Loperamide an antidiarrheal devoid of narcotic cue producing actions Life Sci 16 717 1975
4 Cowen A E & Campbell C B Symptomatic therapy for chronic diarrhoea A comparison of the effects of codeine phosphate and diphenoxylate hydrochloride Med J Aust 1 842 1973
5 Demeulenaere L Verbeke S Muls M & Reyntjens A Loperamide an open multicentre trial and a double blind cross-over comparison with placebo in patients with chronic diarrhoea Curr Ther Res 16 32 1974
6 Dom J Leyman R Schuermans V & Brugmans J Loperamide a novel type of antidiarrheal agent Part 8 Clinical investigation Use of a flexible dosage schedule in a double blind comparison of loperamide with diphenoxylate in 614 patients suffering from acute diarrhea Arzneim Forsch 24 1660 1974
7 Galambos J T Hersh T Schroder S & Wenger J Loperamide a new antidiarrheal agent in the treatment of chronic diarrhea Gastroenterology 70 1026 1976
8 Gianutsos G & Lal H Effects of loperamide haloperidol and methadone in rats trained to discriminate morphine from saline Psychopharmacologia 41 267 1975
9 Karim S M M The effect of polyphloretin phosphate and other compounds on prostaglandin induced diarrhoea in man Ann Acad Med (Singapore) 3 70 1974
10 — Prevention of diarrhoea associated with prostaglandin treatment Presented at the 1st Inter-congress of the Asian Federation of Obstetrics and Gynaecology Singapore April 27–30 1976
11 Lippes J & Hurd M The use of chlorpromazine and Lomotil to prevent and/or reduce the side effects of prostaglandin E_2 used for abortion Contraception 12 569 1975
12 Mainguet P & Fiasse R Double blind placebo-controlled study of loperamide (Imodium®) in chronic diarrhoea caused by ileocolic disease or resection Gut In press 1976
13 Marsboom R Herin V Verstraeten A Vandesteene R & Fransen J Loperamide a novel type of antidiarrheal agent Part 4 Studies on subacute and chronic toxicity and the effect on reproductive processes in rats dogs and rabbits Arzneim Forsch 24 1645 1974
14 McMillin W P A comparison of three anti-diarrhoeals in acute diarrhoea in general practice J Int Med Res 1 226 1973
15 Nelemans F A & Zelvelder W G A double blind placebo-controlled trial of loperamide (Imodium®) in acute diarrhoea Tijdschr Geneesm Ond/J Drug Res 2 54 1976
16 Niemegeers C J E Lenaerts F M & Janssen P A J Loperamide a novel type of antidiarrheal agent Part 1 In vivo oral pharmacology and acute toxicity Comparison with morphine codeine diphenoxylate and difenoxine Arzneim Forsch 24 1633 1974
17 — Loperamide a novel type of antidiarrheal agent Part 2 In vivo parenteral pharmacology and acute toxicity in mice Comparison with morphine codeine and diphenoxylate Arzneim Forsch 24 1636 1974
18 Van Nueten J M Janssen P A J & Fontaine J Loperamide a novel type of antidiarrheal agent Part 3 In vitro studies on the peristaltic reflex and other experiments on isolated tissues Arzneim Forsch 24 1641 1974

19 Pelemans W & Van Trappen G A double blind cross-over comparison of loperamide with diphenoxylate in the symptomatic treatment of chronic diarrhea Gastroenterology 70 1030 1976
20 Rodriguez L The use of loperamide in acute non specific diarrhea Gastroenterology 68 A 117 1975
21 Sharma S D Hale R W & Sato N E Intramuscular (15S)-15 methyl prostaglandin F 2α for mid trimester and missed abortions Obstet Gynecol 46 468 1975
22 Telatar H & Çaglar Ş Results obtained with Lomotil (diphenoxylate hydrochloride) in the treatment of acute and chronic non specific diarrhea and a comparative study of Lomotil and Halquinol Hacettepe Bull Med Surg (Turkey) 2 172 1969
23 Tytgat G N Loperamide and ileostomy output Br Med J 3 489 1975
24 Tytgat G N & Huibregtse K Loperamide and ileostomy output—Placebo-controlled double blind cross-over study Br Med J 2 667 1975
25 Tytgat G N Meuwissen S G M & Huibregtse K Loperamide in the symptomatic control of chronic diarrhoea Double blind placebo-controlled study Ann Clin Res 7 325 1975
26 Verhaegen H De Cree J & Schuermans V Loperamide a novel type of antidiarrheal agent Part 7 Clinical investigation Efficacy and safety of loperamide in patients with severe chronic diarrhea Arzneim Forsch 24 1657 1974
27 Wüster M Schulz R & Herz A Opiates compared with antidiarrheals concerning their affinity to the morphine receptor and action on the isolated guinea pig ileum Naunyn Schmiedebergs Arch Exp Pathol Pharmacol (Suppl) R 35 1976
28 Ylikorkala O Kirkinen P & Järvinen P A Intramuscular administration of 15 methyl prostaglandin $F_2\alpha$ for induction of labour in patients with intrauterine fetal death or an anencephalic fetus Br J Obstet Gynaecol 83 502 1976

Acta Med Scand 202 455–458 1977

The Possible Relation between Postpartum Exacerbation of Hyperthyroidism and Neonatal Thyrotoxicosis

Jørgen Serup and Sten Petersen

From the Departments of Medicine Obstetrics and Gynaecology and Paediatrics Central Hospital Nykøbing Falster Denmark

ABSTRACT Hyperthyroidism is generally considered to be ameliorated during pregnancy, and there appears to be a high incidence of postpartum exacerbation These phenomena have to our knowledge not been related to neonatal thyrotoxicosis, a transient hyperthyroidism seen only in newborns of previous or current hyperthyroid mothers The first of two siblings of a previously thyrotoxic mother had marked symptoms of neonatal thyrotoxicosis and high levels of thyroid hormones The mother had not received antithyroid treatment during her first pregnancy During her next pregnancy she was treated with propylthiouracil from the second trimester This infant had only minimal thyrotoxic signs but almost as high levels of thyroid hormones during the neonatal period as the elder The mother had no signs of postpartum exacerbation but her thyroid hormones were significantly elevated in the postpartum period analogous to the infants Neither the mother nor the infants presented any increase in thyroid stimulating hormone and long acting thyroid stimulator during the hyperthyroid periods The possibility is discussed that postpartum exacerbation of hyperthyroidism and neonatal thyrotoxicosis may be related They could be the result of a changed balance between a thyroid stimulator and an inhibitor after birth

Hyperthyroidism during pregnancy is uncommon with a reported prevalence of about 0 08% (4) It is a general clinical impression that hyperthyroidism is more easily controlled during pregnancy However there does appear to be a relatively high incidence of postpartum exacerbation especially in more severe cases with associated large goitres (4 14 19) The postpartum exacerbation has to our knowledge not been related to the neonatal thyrotoxicosis a transient hyperthyroidism seen only in infants of mothers with previous or current thyrotoxicosis Physical signs of neonatal thyrotoxic disease are often absent at birth but within the first few days or weeks of life the infant develops increasing restlessness diarrhoea tachycardia goitre and exophthalmos The disease is often benign with subsiding symptoms during the following weeks but the infant may die in progressing cardiac failure In a recent review of 75 cases (15) the lethality was 16% Antithyroid therapy with propylthiouracil during pregnancy reduces morbidity and mortality substantially Many observations suggest that a humoral thyrotrophic factor transferred placentally from mother to foetus is responsible for the symptoms in the infant (10)

The present study of a case indicates that the postpartum exacerbation of hyperthyroidism and the neonatal thyrotoxicosis are related The pathogenesis may have common characteristics

METHODS

Total serum thyroxine (T_4) concentration was assayed using the Thyopac® 5 kit from The Radiochemical Centre Amersham England Normal ranges are 65–145 nmol/l in adults and 65–175 nmol/l in pregnant women

Free thyroxine (FT_4) was assayed as normalized thyroxine ratio by the same Thyopac® 5 kit Normal range in adults and pregnant women is 0 88–1 11 rel U

Serum triiodothyronine (T_3) was assayed using The Radiochemical Centre s T_3 kit Normal ranges are 1 3–3 5 nmol/l in adults and 1 5–5 0 nmol/l in pregnant women

Long acting thyroid stimulator (LATS) was assayed using white guinea pigs (11) In this technique LATS values below 1 25 rel U are normal 1 25–1 50 is suspected elevated and above 1 50 is considered significantly pathologic

Thyroid stimulating hormone (TSH) was assayed by

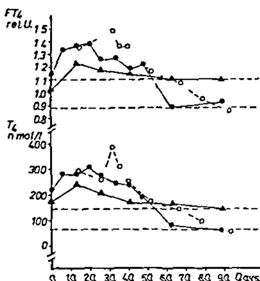


Fig 1 Foetal and maternal serum free thyroxine (FT_4) and total thyroxine (T_4) after birth O O = first infant ●—● = second infant ▲—▲ = mother after second pregnancy - - - = normal range in adults

radioimmune technique using standard 68/38 from The Medical Research Council England Values below 1 9 mU/l are normal

CASE REPORT

The mother A 27 year-old woman who ten years ago (in 966) had undergone partial thyroidectomy indicated by yrotoxic goitre with associated exophthalmos She had nce been well with no thyrotoxic symptoms and no need of antithyroid treatment The exophthalmos persisted and a minimal thyroid enlargement developed again during the years following operation

Non pregnant laboratory data Dec 1974 T_4 155 FT_4 1 13 Nov 1976 T_4 143 FT_4 1 10

First pregnancy The mother developed no thyrotoxic symptoms during the pregnancy and no antithyroid treatment was given She delivered in Feb 1974 a girl at 34 weeks of gestation Birth weight 2050 g Apgar score 10 after 1 min. Physical examination revealed staring eyes but no thyroid enlargement During the first week the infant became hyperkinetic and at three weeks a marked exophthalmos and thyroid enlargement had developed She was treated with phenobarbital The thyrotoxic symptoms gradually subsided during the following 4–6 weeks She was discharged 50 days old in euthyroid state but some exopthalmos persisted The mother presented no specific thyrotoxic symptoms after the delivery

Laboratory data of the infant in the neonatal period are given in Fig 1 LATS could not be detected quantitatively by the method then available but was found qualitatively *TSH was <0 4 mU/l at the age of 2 weeks Maternal T_4* was 182 three days after delivery

Second pregnancy The mother was under strict control in open ward from 18 weeks of gestation She felt well without any thyrotoxic symptoms At 26 weeks of gestation the foetus became hyperkinetic with tachycardia Treatment with propylthiouracil was started indicated by *the foetal symptoms The further medication was based* on maternal laboratory data foetal movements and foetal heart rate (Table I) The mother did not become toxaemic and the circumference of her neck remained unchanged during the medication She gave birth to a boy in Aug 1976 at 37 weeks of gestation Birth weight 2500 g He was asphyxiated with Apgar score 5 after 1 min and score 10 after 10 min The physical examination was normal with no signs of thyrotoxic disease The child was discharged 10 days old He developed no thyrotoxic signs except a slight lid retraction during the neonatal period though his thyroid hormones were transiently elevated (Fig 1) The mother had no subjective or objective thyrotoxic symptoms in the postpartum period The treatment with propylthiouracil was stopped at birth

Cord blood samples T_4 221 FT_4 1 14 T_3 1 6 LATS 0 81 Neonatal laboratory data of the infant are given in Fig 1 TSH 1 6 mU/l at the age of 3 days <0 4 at 7 weeks LATS 0 90 at 2 weeks 0 97 at 4 weeks

Maternal laboratory data after birth are given in Fig 1 TSH<0 4 mU/l 2 and 6 weeks after birth LATS 0 76 and 1 02 T_3 showed when assayed during and after the second pregnancy absolute values and changes parallel to T_4

DISCUSSION

Goitre is twice as common in pregnancy as in the non pregnant state and the thyroid radioiodine uptake is increased (13) The basal metabolic rate is elevated by about 20% during pregnancy This is not due to a hyperthyroid state and the elevation can be attributed to the uterus with its contents and to the increased work of the maternal heart (6) Serum T_4 and T_3 are increased throughout pregnancy due to an elevation in thyroxine binding

Table I *Maternal and foetal parameters during the second pregnancy and treatment with propylthiouracil*

	Gestational age (weeks)				
	18	26	30	34	37
Maternal T_4 (nmol/l)	264	269	120	134	169
Maternal FT_4 (rel U)	1 22	1 25	0 96	1 00	1 02
Maternal T_3 (nmol/l)	>7	>9	–	5 1	–
Maternal LATS (rel U)	0 87	1 56	0 76	0 76	1.20
Foetal heart rate (beats/min)	140	165	140	140	135
Foetal movements	0	+++	+	+	+
Propylthiouracil (mg/24 h)	0	300	75	62 5	62.5

globulin induced by the oestrogens (5) FT_4 remains as in non pregnants and is therefore most valuable for clinical purposes Little is known about free T_3 in pregnancy but it seems to be unaffected like FT_4

After birth maternal T_4 thyroxine binding globulin and thyroid radioiodine uptake return to non pregnant values within the first few days or weeks (13 21) FT_4 remains stable during labor and puerperium No transient hyperthyroid state has been observed during the puerperium following normal pregnancies

Cord blood samples from normal newborns reveal equal or increased T_4 and FT_4 compared to maternal values while T_3 is found to be decreased (1) Following normal pregnancies the newborns present a transient elevation of FT_4 and free T_3 during the first few days accompanied by a more sustained increase in T_4 and T_3 lasting for 4–6 weeks (9) A more pronounced increase in the thyroid hormones is observed in neonatal thyrotoxicosis

The pathogenesis of neonatal thyrotoxicosis is still under discussion It is assumed that a humoral thyroid stimulating factor transferred placentally from mother to foetus is responsible for the symptoms in the infant T_4 T_3 and TSH cross placenta poorly LATS was for a period accepted as the sole pathogenic factor but recently another immunoglobulin G human thyroid stimulating immunoglobulin (HTSI) has been found in infants with neonatal thyrotoxicosis in whom no LATS could be detected (7 10) The immunoglobulins G cross the placenta well HTSI is much better correlated to adult thyrotoxicosis than LATS A thyrotrophic hormone different from chorionic gonadotrophin has been extracted from human placentas (17) However the chorionic thyrotrophin as well as chorionic gonadotrophin itself has only weak thyrotrophic activity Another type of congenital thyrotoxicosis which is genetically determined exists possibly This type is not transient (15)

In the case reported here the first infant had all symptoms of neonatal thyrotoxicosis The second infant born after a pregnancy during which the mother had been treated with propylthiouracil showed only slight lid retraction Nevertheless the infant presented a significant pathologic increase in T_4 and FT_4 almost similar to his elder sister (Fig 1) The treatment with propylthiouracil during pregnancy had excellent clinical effect on the newborn without affecting the levels of T_4 and FT_4 This discrepancy is perhaps due to the peripheral blocking effect of propylthiouracil (12) The mother showed a significant pathologic increase in T_4 and FT_4 after her second pregnancy but like the infant she did not present thyrotoxic symptoms of noticeable degree The increase was transient and parallel to the increases observed in the infants (Fig 1) TSH and LATS were normal in the mother and the new born after the second pregnancy and they could therefore not be responsible for the transient increases in the thyroid hormones

It has been suggested that the amelioration of hyperthyroidism in pregnancy as well as the exacerbation after partus may be due to immunosuppressive mechanisms and the same is sometimes seen in autoimmune thyroiditis (2 3 16) The analogy in the hormonal behaviour of the mother and the infants observed in this case is so striking that it would be reasonable to assume that they were influenced by a common thyroid stimulator originated in pregnancy The appearance of the thyrotoxic symptoms after birth and the transient increases in T_4 and FT_4 after birth may indicate the existence of a common inhibitor which is eliminated faster after the birth than the stimulator The existence of an inhibitor could perhaps also explain the amelioration of maternal thyrotoxicosis in pregnancy Probably the postulated inhibitor has some relation to the placenta The oestrogens influence thyroid physiology in several ways They decrease the basal metabolic rate and the peripheral utilization of thyroid hormone (8 22) The oestrogens are rapidly excreted after birth The exact nature of the thyroid stimulator(s) as well as the postulated inhibitor(s) remain obscure but they are interesting objects of future study

The obstetric and paediatric details of the case are discussed elsewhere (18 20)

REFERENCES

1 Abuid J Klein A H Foley T P & Larsen P R Total and free triiodothyronine and thyroxine in early infancy J Clin Endocrinol Metab 39 263 1974
2 Amino N Miyai K Onishi T Hashimoto T Arai K Ishibashi K & Kumahara Y Transient hypothyroidism after delivery in autoimmune thyroiditis J Clin Endocrinol Metab 42 296 1976
3 Amino N Miyai K Yamamoto T Kuro R Tanaka F Tanizawa O & Kumahara Y Transient recurrence of hyperthyroidism after delivery in Graves disease J Clin Endocrinol Metab 44 130 1977
4 Burrow G N The thyroid gland in pregnancy p 83 Saunders Philadelphia London and Toronto 1972

5 Burrow G N The thyroid in pregnancy Med Clin North Am 59 1089 1975
6 Burwell C S Circulatory adjustments to pregnancy Bull Johns Hopkins Hosp 95 115 1954
7 Dirmikis S M & Munro D S Placental transmission of thyroid stimulating immunoglobulins Br Med J 2 665 1975
8 Engbring N H & Engstrom W W Effects of estrogen and testosterone on circulating thyroid hormone J Clin Endocrinol Metab 19 783 1959
9 Erenberg A Phelps D L Lam R & Fisher D A Total and free thyroid hormone concentrations in the neonatal period Pediatrics 53 211 1974
10 Fisher D A Pathogenesis and therapy of neonatal Graves disease Am J Dis Child 130 133 1976
11 Friis T Long acting thyroid stimulator (LATS) in hyperthyroidism and exophthalmos Dan Med Bull 16 294 1969
12 Geffner D L Azukizawa M & Hershman J M Propylthiouracil blocks extrathyroidal conversion of thyroxine to triiodothyronine and augments thyrotropin secretion in man J Clin Invest 55 224 1975 ,
13 Halnan K E The radioiodine uptake of the human thyroid in pregnancy Clin Sci 17 281 1958
14 Hershman J M McKenzie J M & Selenkow H A Personal communications In Perinatal thyroid physiology and disease (ed D A Fisher & G N Burrow) p 160 Raven Press New York 1975
15 Hollingsworth D R & Mabry C C Congenital Graves disease Am J Dis Child 130 148 1976
16 Nelson J C & Palmer F J A remission of goitrous hypothyroidism during pregnancy J Clin Endocrinol Metab 40 383 1975
17 Nisula B C Morgan F J & Canfild R E Evidence that chorionic gonadotropin has intrinsic thyrotropic activity Biochem Biophys Res Commun 59 86 1974
18 Petersen S & Serup J Neonatal thyrotoxicosis Acta Paediatr Scand In press 1977
19 Selenkow H A Therapeutic considerations for thyrotoxicosis during pregnancy In Perinatal thyroid physiology and disease (ed D A Fisher & G N Burrow) p 145 Raven Press New York 1975
20 Serup J & Petersen S Hyperthyroidism during pregnancy treated with propylthiouracil—the significance of maternal and foetal parameters Acta Obstet Gynecol Scand In press 1977
21 Siersbæk Nielsen K & Molholm Hansen J Variations in plasma thyroxine during labour and early puerperium Acta Endocrinol 60 423 1969
22 Zaninowich A A Thyroxine kinetics during prolonged estrogen administration J Clin Endocrinol Metab 39 949 1973

Acta Med Scand 202 459–462 1977

The Effect of Body Temperature on Thyroid Hormone Levels in Patients with Non-Thyroidal Illness

Jan Gustaf Ljunggren Gunnar Kallner and Monica Tryselius

From the Department of Medicine St Goran s Hospital and the Department of Medicine II Sodersjukhuset Stockholm Sweden

ABSTRACT During studies on the mechanism underlying the low serum T_3 levels in euthyroid patients with various acute and chronic non thyroidal illnesses it became evident that body temperature may be one parameter associated with changes in serum T_3 levels Forty nine hospitalized, euthyroid patients with hyperpyrexia caused by various non thyroidal illnesses were studied The levels of serum T_3 were found to decrease gradually with increasing body temperature T_3 was already below the normal level ±2 S D at a body temperature of around 38°C Such low T_3 levels as were seen at temperatures of above 40°C are observed in thyroid patients only during severe hypothyroidism The levels of T_4 and TSH remained unchanged and within the normal range regardless of body temperature The levels of reverse T_3 in the sera analyzed were found to be unchanged in some cases, while in others they paralleled body temperature It is concluded that the body temperature must be taken into consideration when studying the serum levels of T_3

A significant reduction of serum levels of T_3 (3,5,3 triiodothyronine) despite normal levels of TSH and T_4 (3 5 3 ,5' tetraiodothyronine or thyroxine) has recently been described in euthyroid patients with various acute and chronic non thyroidal illnesses and after starvation or malnutrition (1 3 4 6 7 9 11 12 14 15) Several investigators (3 5 14 15) have observed that changes in T_3 levels are accompanied by close reciprocal changes in serum levels of reverse T_3 (3 3 5 triiodothyronine)

An altered peripheral metabolism of T_4 has been held to be the most likely mechanism behind the changes in hormone levels Thus the alternative pathways for T_4 degradation into the metabolically more active T_3 or to the metabolically inactive reverse T_3 may be a significant regulator of body metabolism The control mechanisms responsible for the hormonal changes and their localization are still unknown The fact that the changes have been observed in a variety of non thyroidal diseases involving different organ systems may indicate that the monodeiodination can be controlled by different mechanisms and can occur at multiple sites It is also possible that the low T_3 levels can be caused by other mechanisms than an increased peripheral conversion of T_4 to reverse T_3 One supporting contention for this possibility is that low T_3 levels have been observed without a reciprocal increase in serum levels of reverse T_3 (14)

During recent years we have seen many euthyroid patients with various non thyroidal disorders and serum T_3 levels within the hypothyroid range despite normal levels of TSH and T_4 In the elucidation of the mechanisms behind these low T_3 levels our attention was drawn to the rapid changes in T_3 levels seen during alterations in body temperature of various etiology

The aim of the present report is to evaluate the relationship between serum T_3 levels and body temperature The results suggest that body temperature is one factor which must be taken into consideration in the elucidation of control mechanisms behind the low T_3 levels seen in various non thyroidal illnesses

PATIENTS AND METHODS

Serum levels of T_3 T_4 reverse T_3 and TSH were analyzed in 49 hospitalized patients with fever caused by various non thyroidal disorders Nineteen of the patients were women with a mean age (±S D) of 62+ [illegible] range 28–87) The mean age (±S D) of t [illegible]

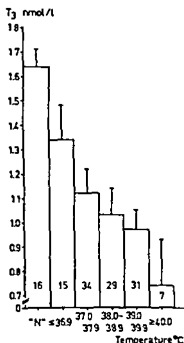


Fig 1 Correlation between T_3 levels (mean ± S E M) and body temperature Samples from group N" were obtained 2–9 months after the fever period The mean age (± S D) in the respective groups was from left to right 51 5±17 0 54 6±22.5 53 9±23 5 53 5±22 6 52 7±22 4 and 53 7±16 8 years

years (range 18–87) The main diagnoses were as follows infection 42 (of the respiratory tract 31 genitourinary tract 6 gastrointestinal tract 3 and meningitis 2) leukemia 2 ocardial infarction 2 sarcoidosis 2 collagen disease 1 samples were taken and body temperature was re ed at the same time Serum was frozen at −20°C un analysis All the samples from each patient were analyzed simultaneously All drugs used in the treatment of the disease were recorded The patients were on a regular hospital diet The study did not influence the treatment of the patients

T_3 and *T_4* were determined by a radioimmunoassay technique recently described (8) the normal level (mean ±S D) for T_3 is 1 77±0 34 nmol/l and for T_4 89±17 nmol/l *Reverse T_3* was determined by A Burger University of Geneva Switzerland the normal level (mean ±S D) is 0 55±0 12 nmol/l *TSH* was analyzed with a commercial kit (Phadebas TSH Pharmacia Diagnostics Uppsala Sweden) the normal level in serum is <5 5 mU/l for women and <7 2 mU/l for men

RESULTS

T_3 levels versus body temperature The association between serum T_3 levels and body temperature in the whole material is presented in Fig 1 It can be seen that the mean T_3 levels gradually decrease with increasing temperature The mean T_3 values are already below the normal level −2 S D at a body temperature of around 38°C (100 4 F) Such low T_3 levels as were seen at temperatures of above 40°C (104°F) occur in thyroid patients only during severe hypothyroidism The mean ages of the different temperature groups given in Fig 1 were found to be similar

Typical results from an individual patient are demonstrated in Fig 2 The reciprocal changes between T_3 levels and body temperature can be seen as well as the parallelism between reverse T_3 levels and body temperature

No parallelism could be seen between changes in T_3 levels and type of disorder with respect to organ

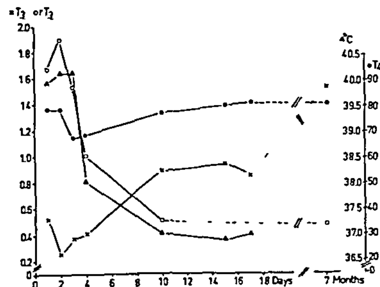


Fig 2 Serum levels of T_3 T_4 and reverse T_3 and body temperature in a 37 year-old woman with respiratory tract infection

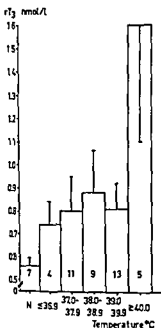


Fig 3 Correlation between reverse T_3 (mean ± S E M) and body temperature

affected administration of drugs or food or other routine parameters determined during the course of the disease A decrease in T_3 levels was found in patients who had completely normal routine function tests from various organs such as liver and kidney Decreased levels were also found in patients without medication and receiving an apparently normal food intake

Reverse T_3 levels versus body temperature Data could not be obtained from all sera The available results are presented in Fig 3 which shows that high levels of reverse T_3 are associated with high body temperature Analysis of data from individual patients revealed a parallelism between body temperature and levels of reverse T_3 in some cases This is demonstrated in Fig 2 In other cases there were no changes of reverse T_3 despite changes in body temperature and T_3 levels The reason for this is not known at present

T_4 levels versus body temperature The association between serum T_4 levels and body temperature in the whole material is presented in Fig 4 As will be seen there were only minor fluctuations within the normal range

TSH levels versus body temperature All TSH levels were within the normal range The mean TSH level (±S D) was 1 71±1 3 mU/l in the samples with the lowest T_3 levels from each patient

DISCUSSION

The decreased T_3 levels seen in the present investigation were obtained from patients with non thyroidal illnesses similar to those previously reported by others (1 3 5) It is thus reasonable to believe that the mechanism(s) behind the decrease are similar Previous investigators have regarded an inhibition of the peripheral conversion of T_4 to T_3 to be the most likely explanation for the decrease in T_3 levels Other possible mechanisms discussed have been a decreased thyroidal secretion increased T_3 distribution volume increased rate of T_3 metabolism or a decreased serum protein binding For ethical reasons we were unable to perform any kinetic studies on our patients with hyperpyrexia or to induce hyperpyrexia in normal human subjects for similar studies

Other investigators have found an association between low T_3 levels and malnutrition starvation old age or drugs These factors can be excluded in the present investigation for the following reasons Malnutrition or starvation could not be the cause of the low T_3 levels found in this investigation Even if one cannot exclude the possibility that the food intake in some patients may have been reduced during the period of extreme hyperpyrexia all the patients received a regular hospital diet A decrease in T_3 levels during starvation has been observed during complete fasting for longer periods (12 15) or during prolonged protein–calorie malnutrition (6)

Old age could not be the cause of the low T_3 levels (13) since the mean age in the different temperature groups was similar and the T_3 levels were within the normal range at normal body temperature

It is unlikely that the decreased T_3 levels were caused by intake of drugs Reduced levels were found in patients receiving no drugs at all In some

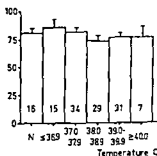


Fig 4 Correlation between T_4 (mean ± S E M) and body temperature

Table I *Mean serum cholesterol and triglyceride values in 26 men taking part in a 90 km cross country ski race*

Range given in parentheses

	Day before	Race day	Day after	2 days after	4 days after
Cholesterol (mg/100 ml)	222 (394–165)	221 (419–162)	209** (371–148)	203** (357–153)	207 * (356–145)
Triglycerides (mmoles/l)	1 60 (2 75–0 98)	1 44 (2 70–1 04)	0 94** (1 39–0 57)	1 18** (1 73–0 69)	1 41 (2 60–0 80)

$^{**}p<0\ 01$

The fatty acid composition of serum lipids was determined by gas chromatography as described by Kirkeby and Bjerkedal (16) The composition of the total serum lipids was determined during the entire observation period whereas the compositions of FFA triglycerides phospholipids and cholesterol esters were determined before and immediately after the race only

Total thyroxine was determined according to Webb (27) and free thyroxine according to Vaerenberg et al (26) Serum osmolality was determined according to Johnson and Hock (12) whole blood Hb by the cyan met hemoglobin method and hematocrit with a Cellocrit 2 centrifuge run at 12 000 *g* for 2 min Serum proteins were determined according to Gornal et al (7)

The statistical evaluations were performed by means of Wilcoxon s signed rank test (30)

RESULTS

Table I shows variations in serum cholesterol and glycerides associated with the 90-km ski race lesterol was unchanged immediately after the but significantly reduced on the following ays including the fourth day after the race The triglycerides showed a tendency to decrease at completion of the race were significantly reduced on the next two days and still slightly lower than before the race on the fourth day afterwards

The average total FFA concentration increased from 621 before the race to 2 164 μEq/l immediately after

The fatty acid composition of serum FFA changed markedly during the race generally towards the composition of ordinary adipose tissue (Table II) The compositon of serum triglycerides showed less pronounced changes mainly a rise in the oleic acid and a decrease in the linoleic acid fraction (Table III)

With regard to the composition of total serum lipids the oleic acid fraction increased during the race whereas the linoleic acid fraction decreased (Table IV) The changes thus reflected the changes in composition of the FFA and the triglycerides described above (Tables II and III) The linoleic acid fraction showed a tendency to remain slightly reduced whereas the arachidonic acid fraction showed a definite increase on the day after the race

Total and free thyroxine were markedly increased at the end of the race but had returned to the pre race levels during the rest of the observation period (Table V)

Serum osmolality and total proteins showed a slight increase during the race whereas blood Hb and hematocrit showed a slight decrease declining further during the following days (Table VI) Although these data are not unequivocal they do

Table II *Fatty acid composition of FFA in 26 men before and immediately after a 90 km cross country ski race and values of adipose tissue biopsies from 25 healthy men*

Mean values with range in parentheses

Fatty acids (%)	Before	After	Adipose tissue
Myristic+ myristoleic	13 0 (3 3–24 0)	5 5 (2 4–8 3)	3 5
Palmitic	19 6 (14 4–24 9)	22 9 (16 1–28 3)	20 8
Palmitoleic	3 7 (2 1–9 2)	6 0 * (3 3–12 9)	7 3
Stearic	11 3 (7 7–15 1)	9 5* (6 0–11 4)	5 3
Oleic	17 9 (14 0–23 5)	30 7 * (17 8–36 8)	45.5
Linoleic	8 1 (4 9–12 7)	11 4* (7 9–15 6)	9.5
Arachidonic	4 1 (3 3–6 6)	1 9* (0 7–3 3)	1 0
Docosapentaenoic	12 1 (4 8–23 1)	4 5 (1 4–12 9)	1 0

$^{*}p<0\ 05$ $^{**}p<0\ 01$

Table III *Fatty acid composition of triglycerides phospholipids and cholesterol esters in 26 men before and immediately after a 90 km cross country ski race*
Mean values with range in parentheses

Fatty acids (%)	Triglycerides		Phospholipids		Cholesterol esters	
	Before	After	Before	After	Before	After
Myristic+ myristoleic	7 4 (2 6–9 8)	5 8 (4 0–11 1)				
Palmitic	25 4 (20 6–38 2)	26 0 (23 1–35 3)	29 4 (22 7–44 0)	28 1 (24 1–32 0)	11 5 (9 1–14 5)	11 4 (9 7–14 0)
Palmitoleic	3 3 (1 8–4 5)	4 5** (3 3–5 6)	1 6 (1 0–3 1)	1 8 (0 8–3 9)	2 7 (1 9–3 6)	2 7 (1 8–3 8)
Stearic	7 1 (4 1–8 9)	6 2 (4 3–9 6)	14 1 (11 1–16 2)	14 6 (12 4–16 6)	1 6 (1 0–2 6)	1 7 (1 2–2 8)
Oleic	30 7 (27 2–33 2)	34 2* (31 4–36 4)	11 2 (10 3–13 7)	11 8 (9 9–16 2)	15 9 (13 8–17 7)	15 7 (13 7–17 2)
Linoleic	17 3 (7 6–23 1)	14 5* (8 0–20 2)	24 5 (20 1–28 7)	24 1 (19 1–28 4)	54 5 (42 6–63 8)	54 9 (45 0–64 4)
Eicosatrienoic			1 5 (0 9–2 5)	1 6 (1 2–2 5)		
Arachidonic	1 1 (0 5–1 6)	1 4 (0 8–2 0)	4 9 (2 9–7 1)	5 5 (3 5–7 3)	5 0 3 8–7 5)	5 0 (2 6–5 9)
Eicosapentaenoic	1 2 (0 7–1 8)	1 0 (0 5–1 3)	1 9 (0 9–3 7)	1 9 (1 1–2 9)	1 9 (1 2–2 8)	1 9 (1 0–2 9)
Docosapentaenoic	2 3 (0 6–3 8)	3 5* (1 3–5 8)	2 3 (1 5–3 2)	2 0 (1 4–3 0)	1 8 (0 6–3 9)	1 8 (0 7–3 3)
Docosahexaenoic			3 7 (1 9–5 9)	3 9 (2 8–5 6)		

*$p<0\ 05$ * $p<0\ 01$

indicate that the observations in Tables I and V cannot be ascribed solely to changes in the state of hydration of plasma volume

DISCUSSION

In accordance with previous studies (10 21) the average energetic cost of the present exercise must have been in the region of 7000 kcal Assuming that the total amount of available carbohydrate including glycogen stores and sugar intake during the race was about 700 g some 500 g of fat must have been mobilized from fat depots to cover the caloric demands during the race The increased fat combustion is reflected by the observation that the fatty acid composition of serum FFA changed markedly towards that of adipose tissue during the race (Table II) The deficiency of available carbohydrate and dependency on fat combustion is further illustrated by the marked increase in β hydroxybutyric acid during the race (21) This is in accordance with current notions of the importance of FFA and ketones as substrate for energy production during exercise (20)

The fact that the triglycerides were only slightly reduced immediately after the race (Table I) disagrees with the observations by Carlson and Mossfeldt (4) in connection with a similar ski race showing a marked fall This discrepancy may partly be ascribed to differences between the two studies for instance with regard to work intensity and fat combustion during the race and times of blood sampling However as reflected by the changes in the fatty acid composition at the end of the race the triglycerides underwent significant changes also in the present study (Table III)

The presented data show that prolonged strenuous exercise in many respects provokes the same pattern of changes in serum lipids as have been observed during serious illness and after severe trauma (1 5 17 18 29) both at the end of exercise at a time of markedly increased demands for fat

Table IV. *Fatty acid composition of total serum lipids in 26 men taking part in a 90 km cross-country ski race*

Mean values with range in parentheses

Fatty acids (%)	Day before	Race day	Day after	2 days after	4 days after
Myristic+ myristoleic	1.2 (0.6–2.7)	1.3 (0.8–2.5)	0.9 (0.6–1.5)	1.1 (0.7–2.9)	1.0 (0.5–2.3)
Palmitic	20.0 (17.9–25.9)	20.6 (18.4–25.0)	20.2 (18.0–23.0)	20.4 (17.6–25.4)	20.0 (17.4–23.6)
Palmitoleic	2.2 (1.6–3.3)	3.0* (2.3–5.3)	2.1 (1.8–2.9)	2.5 (1.6–3.8)	2.3 (1.5–3.7)
Stearic	7.5 (6.4–8.3)	7.5 (6.7–8.9)	7.6 (6.7–9.4)	7.2 (5.9–9.3)	7.6 (6.7–8.9)
Oleic	19.2 (16.0–22.9)	21.4** (18.1–26.2)	18.4 (13.7–26.9)	19.8 (15.2–27.7)	19.6 (14.6–22.8)
Linoleic	36.5 (26.1–40.1)	33.0** (30.6–37.6)	35.7 (25.2–41.8)	35.0* (21.8–40.7)	36.3 (30.2–40.9)
Arachidonic	4.4 (2.9–5.9)	4.4 (3.0–6.2)	5.3** (3.5–7.7)	4.7 (3.2–7.3)	4.4 (2.8–5.9)
Eicosapentaenoic	2.2 (1.0–4.4)	2.2 (1.0–3.1)	2.3 (0.9–4.4)	2.2 (0.8–3.9)	2.0 (1.0–3.6)
Docosapentaenoic	1.8 (0.9–4.1)	2.1 (1.1–4.2)	1.9 (1.0–4.2)	1.8 (0.8–4.0)	1.9 (1.0–3.8)
Docosahexaenoic	2.9 (1.2–4.1)	2.7 (1.5–3.5)	3.2 (1.5–4.6)	2.9 (1.0–4.8)	2.7 (1.4–4.7)

* $p<0.05$ ** $p<0.01$

Table V. *Serum thyroxine and free thyroxine in 26 men taking part in a 90 km cross country ski race*

Mean values with range in parentheses

	Day before	Race day	Day after	2 days after
Thyroxine (µg/100 ml)	7.6 (6.2–9.3)	8.6** (6.5–10.0)	7.6 (5.6–9.2)	7.0 (5.4–9.0)
Free thyroxine (ng/100 ml)	4.7 (2.6–7.9)	7.9*** (1.8–15.8)	5.8 (2.6–9.7)	5.1 (1.9–10.3)

** $p<0.01$ *** $p<0.001$

Table VI. *Serum osmolality and total protein, hematocrit and hemoglobin in 26 men taking part in a 90-km cross country ski race*

Mean values with range in parentheses

	Day before	Race day	Day after	2 days after	4 days after
Osmolality (mOsm/l)	294.2 (290–300)	299.2 (280–308)			
Total protein (g/100 ml)	7.46 (7.1–8.3)	7.66 (6.8–8.5)	7.27 (6.7–8.1)		
Hematocrit (%)	46.1 (39–53)	44.5 (37–52)	44.2 (40–52)	43.9 (40–51)	44.7 (40–48)
Hb (g/100 ml)	15.4 (13.6–16.4)	15.1 (13.0–17.0)	14.7 (13.8–17.0)	14.6 (13.4–16.9)	14.8 (13.7–17.0)

mobilization to cover the energy production and during the following days The delayed changes, including reduction of serum cholesterol and changes in the fatty acid composition of total serum lipids correspond to the changes observed during the days after myocardial infarction (2 5 17 24 29) but are less pronounced Thus taking the pattern of serum lipid changes as an expression of general somatic stress the stress reaction seems to be definitely less pronounced in this type of strenuous exercise than in acute serious illness

The occurrence of increased total and free thyroxine immediately after the race and the subsequent changes in serum lipids indicating an increased β lipoprotein degradation is compatible with the previously presented hypothesis that the accelerated β lipoprotein degradation following acute somatic stress is due to increased thyroid hormonal activity (13)

The increase in free thyroxine immediately after the race was more pronounced than the rise in total thyroxine This seems to indicate a decrease in the thyroxine binding of the serum proteins This assumption is supported by the fact that several of the factors known to influence the affinity of thyroxine to serum proteins were operating during the race such as increased secretion of growth hormone cortisol and catecholamines (3 9, 19 23) increased serum FFA (11) metabolic acidosis (22) and rise in body temperature (8)

REFERENCES

1 Birke G Carlson L A & Liljedahl S D Acta Med Scand 178 337 1965
2 Biorck G Blomquist G & Sievers J Acta Med Scand 156 493 1957
3 Blomstedt B & Einhorn J Metabolism 16 319 1967
4 Carlson L A & Mossfeldt F Acta Physiol Scand 61 51 1964
5 Dodds C & Mills G L Lancet 1 1160 1959
6 Dole V P J Clin Invest 35 150 1956
7 Gornal A G Bardawill C J & David M M J Biol Chem 177 751 1949
8 Harvey R F Lancet 1 208 1971
9 Hays M T & Solomon D H J Clin Invest 48 1114 1969
10 Hedman R Acta Physiol Scand 40 305 1957
11 Hollander C S Scott R L Burgess J A Rabinowitz D Merimee T J & Oppenheimer J H J Clin Endocrinol 27 1219 1967
12 Johnson R B & Hoch H In Standard methods of clinical chemistry vol 5 (ed S Meites) Academic Press New York and London 1965
13 Kirkeby K Acta Med Scand 192 523 1972
14 — Acta Endocrinol (Kbh) 71 62 1972
15 — Acta Endocrinol (Kbh) 71 73 1972
16 Kirkeby K & Bjerkedal I Acta Med Scand 183 143 1968
17 Kirkeby K Hjerman I & Bjerkedal I Acta Med Scand 183 149 1968
18 Man E G Betteher P G Cameron C M & Peters J P J Clin Invest 25 701 1946
19 Oliner L & Ballantine J J J Clin Endocrinol 28 603 1968
20 Paul P In Metabolic adaption to prolonged physical exercise (ed H Howald & J R Poortmans) Birkhauser Basel 1975
21 Refsum H E Tveit B Meen H D & Stromme S B Scand J Clin Lab Invest 32 117 1973
22 Rosin A J & Exton Smith A N Brit Med J 1 16 1964
23 Sundsfjord J A Stromme S B Refsum H E & Aakvaag A Acta Endocrinol (Kbh) (Suppl) 177 184 1973
24 Tibblin G & Cramer K Acta Med Scand 174 451 1963
25 Trout D L Estes Jr E H & Friedberg S R J Lipid Res 1 199 1960
26 Vaerenberg M van Bekaert J Vaerenberg P M & Elewaut A Gesellschaft für Nuclearmedizin Siebente Jahrestagung Zurich 1971
27 Webb A M Standard methods of clinical chemistry vol 7 Academic Press New York and London 1972
28 Webster D Clin Chim Acta 7 277 1962
29 Welin G Nord Med 37 324 1948
30 Wilcoxon F Biometrics Bull 1 80 1945

Acta Med Scand 202 469–473 1977

Lymphocytic Thyroiditis

II The Course of the Disease in Relation to Morphologic Immunologic and Clinical Findings at the Time of Biopsy

Henrik Maagoe Ingermarie Reintoft Hans Ewald Christensen Jørn Simonsen and Erik F Mogensen

From Medical Department M University Hospital and the Institutes of Pathology and Forensic Medicine Odense University Odense Denmark

ABSTRACT Thirty two patients with goitre and lymphocytic thyroiditis were followed for 1½–19 years (average 7) after open surgical biopsy Treatment with thyroid hormone was started when myxoedema was diagnosed Five patients (group A) regained nor[mal] glandular size, remained euthyroid and had ele[v]ated antibody titres Six patients (group B) con[tin]ued to have goitre and elevated antibody titres and remained euthyroid Thirteen patients (group C) developed myxoedema, while 8 (group D) demon[st]rated myxoedema at the time of biopsy The pa[ti]ents in groups C and D had a higher average age [an]d their biopsies showed more marked fibrosis com[pa]red with groups A and B The goitre disappeared [du]ring treatment in 62% of the patients and the [m]icrosomal antibody titre also decreased in them [w]hereas the thyroglobulin antibody titre decreased in [un]treated patients The results indicate that the de[gree] of fibrosis in the thyroid gland is of overall im[po]rtance for the prognosis with regard to glandular [fu]nction It seems evident that the treatment with [thy]roid hormone influences the autoimmune process [so] that the activity decreases

[A] previous publication (13) has pointed out the rela[tion]ship between the morphological immunological [an]d clinical findings in a series of 32 patients with [goi]tre and lymphocytic thyroiditis The present [pap]er describes the course of the goitre the func[tio]n of the thyroid gland and the thyroid antibodies [in p]lasma and discusses how much the morphology [pre]dicts about the disease and its further course [whe]n thyroid hormone substitution has begun This [is o]f special interest as the group of patients may be [reg]arded as homogeneous even with regard to the [futu]re course of the disease

METHODS

The subjects are the 32 patients described earlier (13) After open surgical biopsy the sections were evaluated without knowledge of the clinical data. The presence of PAS-positive material number of lymphocytes and plasma cells as well as the degree of fibrosis and amount of reticulin fibres were estimated semiquantitatively (13) The occurrence of epithelial changes with metaplasia to Askanazy cells was noted but not graded The subjects were followed in the Out Patient Department at monthly intervals for 1½–19 years (average 7) Hormone therapy was instituted only when hypothyroidism became mani fest as judged from the level of thyroxine iodine in plasma and the result of the T_3 resin uptake test in plasma.

T_4 I (normal value 3 2–7 6 μg/100 ml plasma) and T_3-resin uptake tests (normal value 82–108 %) in plasma were estimated by the Tetrasorb 125 and "Triosorb 125 methods respectively TSH (normal value <3 mU/l plasma) in plasma has been estimated in recent years by a radioimmunoassay technique The thyroglobulin anti bodies and the microsomal thyroid antibodies were esti mated by passive haemagglutination test as well as by con ventional technique with a complement fixation test The size of the thyroid gland was estimated by digital palpa tion

The statistical evaluations have been made with the χ^2 test with Yate s correction Mann Whitney s *U* test for unpaired data and Wilcoxon s signed rank test for paired data (23)

RESULTS

The clinical course of the goitre thyroid function and antibody titres may be deduced from Fig 1 At the time of biopsy all patients had goitre 24 were euthyroid and 8 suffered from myxoedema (group D) Twenty two patients demonstrated thyro globulin antibodies and 17 microsomal antibodies 13 exhibiting both types of antibodies [illegible] ither kind

The 8 patients with my[illegible] ma f[illegible] e of

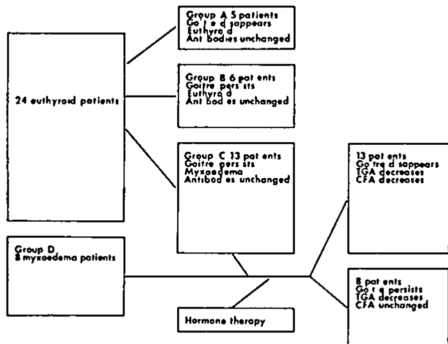


Fig 1 The course of the disease in 32 patients with goitre and lymphocytic thyroiditis *TGA*=thyroglobulin antibodies *CFA*=complement fixing antibodies

biopsy (group D) were treated as soon as the diagnosis had been established The 24 euthyroid patients were subdivided into three groups (A B and C) according to the changes in the size and function of the gland

Group A 5 patients observed for 6–10 years An average of 1½ years passed before the size of the gland was normal These patients did not receive treatment However at the end of the observation period 2 patients showed a slight elevation of the TSH in plasma probably as a sign of incipient thyroid insufficiency (Table I) The titres for the antibodies did not change significantly

Group B 6 patients observed for 5–13 years The goitre persisted and they all remained euthyroid but one patient demonstrated a slight elevation of the TSH level in plasma at the end of the observation period (Table I) No significant changes in the antibody titres were found

Group C 13 patients who in the average course of 1½ years developed myxoedema In the preceding phase the goitre and the antibody titres remained unchanged

The 21 myxoedematous patients from groups C and D were followed during the substitution therapy for 1–9 years (average 5) The goitre disappeared completely in 13 patients and remained almost unaltered in 8 (Fig 1) The level of the thyroglobulin antibodies decreased ($p<0.02$) during hormone therapy regardless of the size of the gland while microsomal antibodies were diminished only in group in which the goitre disappeared ($p<0$ [illegible] The values of T_4 I T_3 resin uptake and TSH given in Table I

With regard to the clinical immunological morphological findings groups A and B were similar and no significant differences were covered between groups C and D either The similarities and differences between the two new m groups A+B and C+D are listed in Table II The morphological findings showed the most mark difference It must be concluded that patients w manifest hypothyroidism have more pronoun fibrosis ($p<0.001$) In these patients the reticu content is augmented and the total glandular struction as measured by the PAS reaction greater than in the rest of the patients Less diff ence was found in plasma cell infiltration There were no significant differences in the antibody titr at the end of the observation period At the time biopsy euthyroid patients in group C showed mo fibrosis than the group A+B ($p<0.02$) The patie in group C+D are on an average 10 years older th those in group A+B ($p<0.05$)

The patients in groups C and D who during the hormone therapy showed disappearance of the goitre did not at the time of biopsy differ in a way from the patients with persistent goitre

Table I *Function of the thyroid gland at the end of the follow up period*

Pat no	T_4-I (μg/100 ml)	T_3 resin uptake (%)	TSH (mU/l)
Group A			
1	6 1	79	3 0
2	3 6	84	4 0
3	5 7	88	10
4	7 7	76	2 7
5	7 3	98	1 8
Group B			
6	6 2	98	3 5
7	3 6	106	8 6
8	4 4	76	15 4
9	5 1	84	0 8
10	5 1	85	11 5
11	4 4	81	5 5
Group C+D (goitre disappears)			
12	3 2	83	89
13	1 4	71	40
14	2 8	73	39
15	1 0	75	11 9
16	–	74	–
17	–	76	–
18	1 5	80	1 1
19	1 5	74	5 5
20	4 7	83	26
21	0 8	79	4 8
22	0 6	79	30
23	–	92	–
24	4 5	93	10 8
Group C+D (goitre persists)			
25	1 8	90	35
6	3 5	83	2 7
	1 5	81	25
	2 0	73	11 5
	1 6	85	66
	1 3	75	69
1	3 4	85	3 0
2	2 5	78	75

DISCUSSION

lassically a patient with Hashimoto s struma develops myxoedema after some years The patients in groups C and D fulfil this criterion They show significantly more fibrosis of the glandular tissue than the patients in groups A and B There seems to be good evidence that these morphological findings are of significant importance for the course of thyroid function However it seems remarkable that the type and severity of cell infiltration as well as the antibody titres play only a minor role in this respect

After thyroid hormone therapy of the myxoedematous patients the goitre disappeared in 13 and persisted in 8 It is well known that both cortisone treatment (3 22) and thyroid hormone treatment (1 6 8 10 14 17 18 21) lead to a reduction in the size of the thyroid gland in at least 75% of both euthyroid and myxoedematous patients However there is one contradictory report (22) A possible explanation of the reduction of the glandular tissue could be a suppression of TSH in plasma—an explanation which does not hold good in the present patients Two of the eight patients with persistent goitre were optimally treated as judged from the TSH plasma level and about half of the 13 patients with normalization of glandular size and treated with thyroid hormone had plasma TSH levels higher than 10 mU/l It has been reported that pronounced fibrosis should suffice for the persistence of goitre in spite of substitution therapy but this has not been proved in the present study population

During treatment a decrease was observed in thyroglobulin antibodies in both groups of patients But a fall in the complement fixating antibody titre was only seen in patients with disappearance of the goitre It is well known that thyroidectomy (5) and cortisone treatment (3 22) may lead to abatement of the titres for circulating thyroid antibodies Some investigators (10 16) have found a similar effect of thyroid hormone in euthyroid as well as in myxoedematous patients while others do not confirm this observation (9 12 17 18 24) In this connection the role of thyroid hormone as an immunosuppressive agent has been discussed Although it must be recognized that the type of inflammatory reaction correlates better to the pattern of histological findings in delayed hypersensitivity reactions than

able II *Differences and similarities between groups A+B and C+D*
he significant changes were on the whole more pronounced in group C+D
GA=thyroglobulin antibodies CFA=complement fixing antibodies
S=not significant

ge	Duration of the disease	TGA	CFA	PAS staining	Lymphocytes	Plasma cells	Reticulin	Fibrosis
<0 05	NS	NS	NS	$p<0\ 002$	NS	$p<0\ 05$	$p<0\ 002$	$p<0\ 001$

it does to the presence of thyroid antibodies (15 19) our results are in good accordance with the findings of Persson (18) based on repeated biopsies This is also in accordance with the theory concerning early proliferative and late fibrotic stages of the disease (11) The demonstration that treatment with thyroid hormone does not diminish the progression of the disease to myxoedema (12 17 21) is not entirely at odds with this theory because fibrosis in the present material proved to be decisive for the development of myxoedema However, some investigators using repeated biopsies found only slight changes in the morphology of the glandular tissue in cases with long duration of the disease (16 25)

The present patients form a homogeneous group from the beginning of the observation since all of them were suffering from goitre and lymphocytic thyroiditis In group A the size of the gland became normal but the antibody titres were unchanged and the patients remained euthyroid Without knowledge of the previous goitre these patients might be designated as cases of autoimmune symptomless thyroiditis In group B the patients in spite of a long follow up period demonstrated unchanged antibody titres and goitre and did not develop myxoedema Both in group A and in group B several patients had increased plasma TSH probably as a sign of incipient glandular insufficiency Such patients are predicted to run an especially high risk of developing myxoedema (7)

The difference between groups A+B and C+D is quantitative The latter group did show a slightly higher average age and more pronounced glandular fibrosis Bastenie et al (2) in particular have studied quantitative morphologic expressions as significant for different types of disease whereas others (10 11 26) consider that the changes shown are different manifestations of the same disease In connection with the difficulty of interpreting the morphological findings it should be stressed that juvenile thyroiditis has very little fibrosis and a corresponding low tendency to development of myxoedema (4 21) In accordance with this experimental autoimmune thyroiditis also shows different types of course in terms of morphology (20)

ACKNOWLEDGEMENT

This study was supported by the local Research Foundation (Fyns amts regionale forskningsfond)

REFERENCES

1 Astwood E B Cassidy C E & Auerbach G D Treatment of goiter and thyroid nodulus with thyroid JAMA 174 459 1960
2 Bastenie P A Ermans A M & Nève P Focal lymphocytic thyroiditis in simple goitre In Thyroiditis and thyroid function (ed P A Bastenie & A M Ermans) pp 143–158 Pergamon Press New York 1972
3 Blizzard R M Hung W Chandler R W Aceto T Kyle M & Winship T Hashimoto s thyroiditis N Engl J Med 267 1015 1962
4 Clayton G W & Johnson C M Struma lymphomatosa in children J Pediatr 57 410 1960
5 Doniach D & Roitt I M Auto-immunity in Hashimoto s disease and its implications J Clin Endocrinol 17 1293 1957
6 Furr W E & Crile G Struma lymphomatosa Clinical manifestations and response to therapy J Clin Endocrinol 14 79 1954
7 Gordin A & Lamberg B A Natural course of symptomless autoimmune thyroiditis Lancet 2 1234 1975
8 Heimann P Treatment of thyroiditis Acta Med Scand 187 323 1970
9 Jeske W & Gorowski T Effect of T_3 treatment on antibodies in Hashimoto s disease Pol Arch Med Wewn 47 493 1971
10 LeBoeuf G & Ducharme J R Thyroiditis in children Pediatr Clin North Am 12 19 1966
11 Lindsay S Dailey M E Friedlander J Yee G & Soley M N Chronic thyroiditis Clinical and pathologic study of 354 patients J Clin Endocrinol 12 1578 1952
12 Ling S M Solomon A K Weitzman J J Reid G B Costin G & Landing B H Euthyroid goiter in children Correlation of needle biopsy with other clinical and laboratory findings in chronic lymphocytic thyroiditis and simple goiter Pediatrics 44 695 1969
13 Maagøe H Reintoft I Christensen H E Simonsen J & Mogensen E F Lymphocytic thyroiditis I Correlation between morphological immunological and clinical findings Acta Med Scand 201 299 1977
14 McConahey W M Woolner L B Black B M & Keating F R Effect of desiccated thyroid in lymphocytic (Hashimoto s) thyroiditis J Clin Endocrinol 19 45 1959
15 McMaster P R B Lerner E M & Exum E D The relationship of delayed hypersensitivity and circulating antibody to experimental allergic thyroiditis in inbred guinea pig J Exp Med 113 611 1961
16 Owen S G & Smart G A Thyroid antibodies in myxoedema. Lancet 2 1034 1958
17 Papapetrou P D Lazarus J H McSween R N M & Harden R M Long term treatment of Hashimoto s thyroiditis with thyroxine Lancet 2 1045 1972
18 Persson P S Cytodiagnosis of thyroiditis Acta Med Scand (Suppl) 483 40–44 64 70–75 1968
19 Rose N R Kite J H & Doebbler T K Experi-

mental autoimmune thyroiditis In Mechanism of cell and tissue damage produced by immune reactions (ed P Grabar & P Miescher) p 161 Schwabe Basel 1962

20 Rose N R Skelton F R Kite J H & Witebsky E Experimental thyroiditis in the rhesus monkey 3 Course of the disease Clin Exp Immunol 1 171 1966

21 Saxena K M & Crawford J D Juvenile lymphocytic thyroiditis Pediatrics 30 917 1962

22 Shuhei I Tamura T & Nishikawa M Effect of desiccated thyroid prednisolone and chloroquine on goiter and antibody titer in chronic thyroiditis Metabolism 17 317 1968

23 Siegel S Non parametric statistics pp 202–213 McGraw Hill New York 1956

24 Skanse B & Nilsson S B Thyroid antibodies in hypothyroidism Acta Med Scand 170 461 1961

25 Vickery A L & Hamlin E Struma lymphomatosa (Hashimoto s thyroiditis) observations or repeated biopsies in sixteen patients N Engl J Med 264 226 1961

26 Woolner L B McConahey W M & Bearhs O H Struma lymphomatosa (Hashimoto s thyroiditis) and related thyroidal disorders J Clin Endocrinol 19 53 1959

Acta Med Scand 202 475–480 1977

Thyroid Function in Malignant Lymphoma

Lotte Maagaard Brinckmeyer Anne Marie Worm and Nis I Nissen

From the Department of Internal Medicine The Finsen Institute Copenhagen Denmark

ABSTRACT Thyroid function was studied in 36 patients with various stages of malignant lymphoma Stage IVB patients exhibited characteristic changes in thyroid biochemistry in the form of lowered triiodothyronine (T_3) and elevated free thyroxine (FT_4), but normal thyroxine Moreover the concentration of thyroxine binding prealbumin and albumin was lowered, whereas thyroxine binding globulin was normal Thyroid stimulating hormone was slightly elevated but showed a normal increase after administration of thyrotrophin releasing hormone Patients with less extensive disease differed only slightly from the controls The results agree with previous studies of patients suffering from other chronic diseases The mechanisms underlying the hormonal changes have been only partially elucidated When investigating patients with disseminated malignant disease for thyroid disease, the above mentioned changes in thyroid biochemistry must be borne in mind Single analyses of FT_4 and T_3 may give rise to a false assumption of hyper or hypothyroid states in patients who are in fact euthyroid

Patients with malignant lymphoma often exhibit systemic symptoms and signs such as sweating weight loss low grade fever and tachycardia In accordance with these clinical signs of hypermetabolism early studies have demonstrated a common occurrence of an elevated basal metabolic rate (26 28) More detailed descriptions of thyroid function including determinations of thyroxine (T_4) free thyroxine (FT_4) triiodothyronine (T_3) and thyroid stimulating hormone (TSH) have recently been reported in patients with other chronic diseases (2 8 10 16 20) but only sporadically in patients with malignant lymphoma

We therefore tried to elucidate several aspects of thyroid function in a group of patients with malignant lymphoma The results were related to the stage of their disease and to various organ functions

STUDY POPULATION AND METHODS

The study comprises all patients with Hodgkin s disease lymphosarcoma or reticulosarcoma but with no other chronic diseases referred to us during a 4 month period around the turn of 1974

According to a number of staging procedures in [illegible] X rays of the chest and stomach i v pyelogr of lymphangiography percutaneous liver biopsy and bone marrow biopsy the extent of the disease was classified into one of the following stages I=involvement of lymph nodes in one region II=involvement of lymph nodes in two or more regions on the same side of the diaphragm III involvement of lymph nodes on both sides of the diaphragm IV=involvement of extralymphatic structures e g lung liver and/or bone marrow Furthermore to each stage designation an A or a B was added to indicate whether systemic symptoms such as fever sweating and/or weight loss were absent or present

Table I gives the sex age and stage for the 36 patients None had a personal or family history of thyroid diseases There was no case of primary thyroid lymphomas

The control group comprised healthy clinically euthyroid persons aged 20–74 years with the same sex ratio none of whom was on oral contraceptive or other medication

All investigations were performed at the time of primary staging before instituting treatment of the basic disease

T_3 and TSH were determined by radioimmunoassay using a double antibody technique (9 17) thyroxine binding globulin (TBG) by the method of Nielsen et al (19) T_4 was determined by a competitive protein binding technique (18) FT_4 on the basis of the ratio between free dialysable and protein bound T_4 (25) and thyroxine binding prealbumin (TBPA) by electroimmunodiffusion (13) In the patients but not in the controls TSH was measured 30 min after i v administration of 200 μg thyrotrophin releasing hormone *(TRH)=TRH test* All the above mentioned analyses were performed by Medicin k Laboratorium Copenhagen as some of the methods had not at that time been fully established as a routine in our hospital All other chemical analyses were carried out in the Department of Clinical Chemistry of the Finsen Institute

In the statistical analysis of the results Student s *t* test was used As this test presupposes a normal distribution of the material the results were analysed also by a non parametric test (Wilcoxon s two-sample test) The signifi

Table I *Thyroid function tests and other clinical data in 36 patients with malignant lymphoma*

Case no	Sex	Age (y)	Stage	T_3 (ng/100 ml)	FT_4 (pmol/l)	T_4 (nmol/l)	TBG (U/l)	TBPA (μmol/l)	Albumin (g/l)	TSH (mU/l)
1	♂	32	I A	145	23 2	98	108	–	46	–
2	♂	37	I A	156	26 2	118	121	4 1	49	1 9
3	♂	57	I A	94	32 2	108	117	4 4	40	0 4
4	♂	68	I A	98	36 7	92	103	–	36	5 7
5	♂	35	II A	135	28 2	112	109	–	45	0 6
6	♂	63	II A	120	18 0	78	117	–	38	5 8
7	♀	25	II A	118	27 3	115	107	3 9	36	2 0
8	♀	51	II A	127	29 3	113	115	2 5	–	2 7
9	♂	23	II A	239	28 8	131	114	2 5	44	0 6
10	♂	29	II B	119	30 2	105	115	2 5	38	2 7
11	♀	26	III A	216	32 1	122	103	3 3	–	1 5
12	♀	63	III A	129	23 5	115	115	3 3	37	2 5
13	♂	21	III B	133	24 0	104	131	–	38	2 0
14	♂	47	III B	209	33 5	100	109	4 9	46	1 2
15	♀	29	III B	122	31 2	–	119	–	40	2 8
16	♀	29	III B	107	26 2	127	–	–	42	1 5
17	♂	60	IV A	130	31 8	132	87	3 3	38	0 7
18	♀	71	IV A	139	33 7	122	137	–	40	1 6
19	♀	67	IV B	96	31 7	104	121	1 6	28	2 9
20	♀	70	IV B	32	43 3	73	–	–	28	10 0
21	♀	20	IV B	75	75 7	142	–	–	28	1 5
22	♀	72	IV B	72	40 2	103	109	2 1	–	2 [illegible]
23	♀	63	IV B	19	94 1	110	–	–	26	3 [illegible]
24	♂	88	IV B	51	36 2	84	–	–	37	2 1
25	♂	48	IV B	24	63 7	87	–	–	27	1 3
26	♂	63	IV B	45	74 3	122	–	–	40	2 9
27	♂	48	IV B	75	25 5	86	79	–	40	10 8
28	♀	39	IV B	130	46 5	171	147	–	39	1 1
29	♂	45	IV B	78	25 8	108	–	–	35	1 9
30	♀	62	IV B	29	68 2	103	74	–	35	3 1
31	♂	54	IV B	56	34 4	76	52	3 1	30	0 4
32	♀	67	IV B	132	52 2	165	134	1 8	30	3 4
33	♂	81	IV B	67	43 5	128	93	2 1	30	1 7
	♀	46	IV B	58	47 2	110	104	1 4	19	2 0
)	♀	61	IV B	39	61 8	154	–	–	–	2 3
	♀	13	IV B	97	39 6	112	113	2 1	36	2.0

cance levels coincided Moreover the results were assessed mutually by correlation analysis (Spearman s rank correlation coefficient)

RESULTS

For patients in stages I II and III the results showed no significant differences from those in the control group unlike the results for stage IV patients Neither was there any difference in the results between patients with and without systemic symptoms and signs in stages I–III while this appeared to be so for stage IV patients In the final analysis of the material therefore we divided the patients into two groups group I comprising patients in stages I II III A and B and IV A while group II comprises patients in stage IV B

The individual findings for all patients are listed in Table I the results and statistical calculations in Table II The latter also gives the results of the statistical comparison of group I/control group group II/control group and group I/group II

T_3 proved greatly reduced in group II patients as compared with group I patients and the control group ($p<0\ 001$) and the majority of the group II patients had T_3 values at a level which is characteristic of patients having hypothyroidism (7) In group I the mean T_3 was higher than in the control group ($p<0\ 001$) because three patients had unusually high levels These 3 patients did not exhibit clinical signs of T_3-thyrotoxicosis and their euthyroidism was furthermore proved by a normal TRH response The T_3 levels found in the various groups are further illustrated in Fig 1

FT_4 was significantly elevated in group II as com-

test H	Creatinine (µmol/l)	SGOT (U/l)	ALP (U/l)	Liver biopsy
	90	16	64	Steatosis
	104	16	56	Normal
	99	14	62	Normal
	104	16	34	Normal
	90	38	52	Steatosis
	–	19	64	Normal
	70	25	43	Normal
	88	31	112	Steatosis
	66	12	56	Normal
	97	21	77	Steatosis
	61	14	64	Normal
	85	17	116	Steatosis
	85	19	128	Normal
	140	37	82	Steatosis
	71	32	51	Normal
	–	23	51	Normal
	96	22	68	Mal infil
	87	36	63	Steatosis
	74	14	96	Normal
	112	13	94	–
	56	22	236	Normal
	88	22	86	Normal
	115	16	85	–
	210	18	47	–
	–	71	511	–
	75	21	81	Mal infil
	75	14	82	–
	66	32	112	Mal infil
	71	25	256	Normal
	133	51	284	–
	–	19	65	Mal infil
	90	20	79	Mal infil
	76	22	68	Normal
	64	15	218	Normal
	88	41	92	Steatosis
	134	70	78	Mal infil

pared with group I ($p<0.001$) and also as compared with the control group ($p<0.001$). On the other hand there was no significant difference between group I and the control group. The FT_4 findings in the individual groups are also plotted graphically in Fig. 2 which shows that most patients of group II had FT_4 levels which are usually characteristic of hyperthyroidism (25).

T_4 did not differ significantly from the control group in either group I or group II, neither did it do so between the latter two groups.

The protein binding of the thyroid hormones was elucidated by determining TBG, TBPA and albumin. There was no significant difference in the TBG concentration either between groups I and II or between each of these and the control group. On the other hand, there was a definite reduction of both TBPA and albumin in group II compared with the control group ($p<0.001$). There was also a significant reduction of TBPA ($p<0.001$) but not of albumin in group I compared with the control group. Comparisons between the two groups of patients showed both TBPA and albumin to be lower in group II ($p<0.005$ and $p<0.001$).

The relationship between the thyroid and pituitary glands was elucidated by determination of the TSH. The TSH level was slightly increased in group I ($p<0.01$) as well as in group II ($p<0.005$) compared with the control group, but there were no significant intergroup differences. Pituitary function was elucidated by the TRH test. Both groups showed a normal increase in TSH following administration of TRH ($\Delta TSH>2$ mU/l (14)).

Renal function, elucidated by serum creatinine, proved normal in both groups.

To ascertain a possible involvement of the liver by the neoplastic disease, the serum concentrations of GOT and alkaline phosphatase (ALP) were investigated. In group II, SGOT was slightly in-

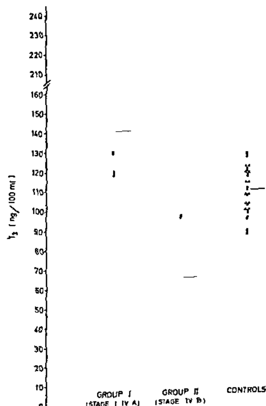


Fig. 1. Serum T_3 concentrations in patients with malignant lymphoma and in control subjects. (The solid line represents the mean value.)

Table II *Results and statistical significance of the differences*

	T_3 (ng/100 ml)	FT_4 (pmol/l)	T_4 (nmol/l)	TBG (U/l)	TBPA (μmol/l)	Albumin (g/l)	TSH (mU/l)
Group I (stages I–IV A)							
Mean	141	28 7	111	113	3 5	41	2 1
S D	40 4	4 6	14 3	11 2	0 8	4 0	1 6
n	18	18	17	17	10	16	17
Group II (stage IV B)							
Mean	65	50 2	113	103	2 0	32	3 0
S D	33 2	19 0	29 1	28 8	0 5	5 9	2 8
n	18	18	18	10	7	16	18
Controls							
Mean	110	27 4	99	120	5 1	42	1 3
S D	17 5	5 0	22 9	30 2	0 7	2 4	0 7
n	36	33	32	34	21	22	32
Group I/group II							
t	6 14	4 68	0 25	1 39	3 96	5 06	1 19
p	<0 001	<0 001	NS	NS	<0 005	<0 001	NS
Group I/controls							
t	3 90	0 92	1 95	0 84	5 70	1 36	2 66
p	<0 001	NS	NS	NS	<0 001	NS	<0 01
Group II/controls							
t	6 56	6 56	1 86	1 60	10 85	7 55	3 44
p	<0 001	<0 001	NS	NS	<0 001	<0 001	<0 00[illegible]

creased in relation to the control group ($p<0.01$) but there was no significant difference between group II and group I AIP was greatly increased in group II both in relation to the control group ($p<0.001$) and to group I (p 0 01)

istolog al examination of the liver biopsies re steatosis or malignant infiltrates in the liver 18 patients of group I and 6/12 of group II All er patients had normal liver biopsies Comparisons within group I or group II of all the parameters listed in Table II did not reveal any significant differences between patients with and without histologically confirmed hepatic changes

In group II there was an inverse correlation between FT_4 and T_3 (Spearman's rank correlation coefficient −0 470 $p<0.05$) while this did not apply to group I

All the TBPA albumin TSH and alkaline phosphatase values for group II patients were related to the FT_4 and T_3 values in the same patient but no significant correlation was found ($p>0.1$)

At the time of the investigation 5 patients of group I were receiving medication containing oestrogen (2 patients) phenylbutazone (1 patient) and phenytoin (2 patients) Within group II three patients were receiving oestrogen (1 patient) salicylate (2 patients) and phenylbutazone (1 patient) Excluding these patients from the material did not alter the above results and therefore all were included

DISCUSSION

It was demonstrated in the present study that patients with advanced malignant lymphoma (stage IV) and systemic symptoms exhibit characteristic changes in thyroid biochemistry viz reduced T_3 and elevated FT_4 but normal T_4 But if the basic disease is in a localized stage the mean concentration of the named thyroid hormones with one exception (elevated T_3) did not differ significantly from the normal levels

Similar findings have been reported previously with a few variations in clinically euthyroid patients suffering from other chronic diseases such as hepatic cirrhosis ulcerative colitis decompensated cardiac and pulmonary diseases renal diseases infections and neoplastic diseases (1 2 3 8 10 11 15 16 20 21) Despite numerous investigations the mechanisms underlying the hormonal changes have not yet been clarified

An elevated FT_4 is a typical finding in hyperthyroidism due to an increased secretion of T_4 and an altered T_4 protein binding in the serum In serum T_4 is bound to TBG (75%) TBPA (15%)

TRH test TSH (mU/l)	Creatinine (μmol/l)	SGOT (U/l)	ALP (U/l)
6	89	23	69
4	18 9	8 5	25 6
5	16	18	18
7	95	28	143
6	38 7	18 3	117 3
5	16	18	18
	89	19	51
	12 8	4 7	15 4
	40	38	40
16	0 57	1 15	2 60
3	NS	NS	<0 01
	0 13	1 80	3 32
	NS	NS	<0 005
	0 99	2 75	4 90
	NS	<0 01	<0 001

and albumin (10%) (29) Changes in the concentration of these proteins may entail a shift in the ratio between free and bound T_4

In our study and in several similar ones T_4 proved normal or slightly reduced (2 6 8 10 16 30) which argues against an increased T_4 secretion being the cause of the increased FT_4 concentration This is further supported by the demonstration of a normal ^{131}I thyroid clearance and 24 hour ^{131}I uptake in a corresponding group of patients having elevated FT_4 (3)

We found a normal TBG level in agreement with previous studies (6 10 21) The demonstration of a reduced TBPA and albumin might be a reasonable explanation of the increased FT_4 (3 21) but several findings indicate that this cannot be the sole cause For instance in our study there was no correlation between the FT_4 level and the concentration of TBPA or albumin in one and the same patient Neither experimental total removal of TBPA from the serum by immune adsorption (29) nor mathematically calculated total elimination of TBPA and albumin (5 12) elicited an increase in FT_4 like that observed Lastly sequential measurements of TBPA and FT_4 in experimental clinical infections have shown a chronological dissociation between the decrease in TBPA and the increase in FT_4 Thus factors other than changes in TBPA concentration contribute to regulate the concentration of free hormone (15) so that it is impossible to point out a single factor as the most likely cause of the elevated FT_4 in our patients

The reduced level of T_3 might theoretically be due to an altered protein concentration a reduced secretion from the thyroid gland or a reduced peripheral conversion of T_4 into T_3

Like T_4 the greater part of T_3 in the serum is bound to TBG TBPA and albumin (12) A reduced concentration of one or more of these proteins might contribute to the low T_3 level demonstrated but the failure to find a simultaneously reduced T_4 speaks against this possibility Considering the normal T_4 secretion it is moreover difficult to imagine an isolated reduction of T_3 secretion from the thyroid gland especially as the serum T_3 response following administration of TRH seems to be normal (8 10) Pituitary function assessed by an increase in TSH after administration of TRH was also normal in our study as well as in studies by others (8 10) However these findings do not definitely rule out hypothalamic or pituitary dysfunction (22) We and others have found a slightly but

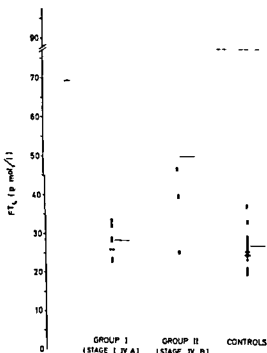


Fig 2 Serum FT_4 concentrations in patients with malignant lymphoma and in control subjects (The solid line represents the mean value)

significantly elevated basal serum level of TSH (2 10 20) The role of the T_3 concentration in this respect is doubtful partly because there was no correlation between the changes in TSH and in T_3 in the individual patient and partly because most T_3 values were clearly in a hypothyroid range which in primary hypothyroidism is accompanied by higher TSH values than those observed (14) In this connection however it must be admitted that the influence of the combination reduced T_3/elevated FT_4 upon TSH secretion is uncertain

The T_3 level in the serum declines with advancing age (2 24) but this does not explain the low T_3 levels found in the present study as the control group was comparable in age with the patients A recent study by Bermudez et al (2) also showed no correlation between the T_3 in patients with non thyroidal disease and the levels expected according to their age

The most probable cause of the lowered T_3 concentration is reduced peripheral conversion of T_4 into T_3 (20) and instead increased formation of an inactive hormone the so-called reverse T_3 (6)

T_4 conversion which seems to be an important source of T_3 (4 23 27) may take place in various tissues presumably mainly in the liver (20) Study ing patients with hepatic cirrhosis Nomura et al (20) demonstrated a significant reduction in the conversion of T_4 into T_3 they also found elevated FT_4 owered T_3 elevated TSH and normal T_4—the me pattern as we obtained Nomura et al concluded that inhibited peripheral conversion of T_4 into T_3 in the liver is characteristic of patients with hepatic cirrhosis but that this could not explain the abnormal T_3 levels in other non thyroidal diseases Indeed we did not find a correlation between T_3 or FT_4 and biochemical or histological signs of hepatic involvement This leaves us with the hypothesis of Bermudez et al (2) that the low T_3 levels should be attributed to the catabolic state which accompanies chronic illness rather than to specific disease entities

In conclusion it should be emphasized that patients with disseminated malignant disease exhibit changes in thyroid chemistry which must be taken into consideration when the patients are being investigated for thyroid disease Single analyses of FT_4 and T_3 may give rise to a false assumption of hyper or hypothyroid states in patients who are in fact euthyroid

REFERENCES

1 Bellabarba D Inada M Varsano-Aharon N & Sterling K J Clin Endocrinol 28 1023 1968
2 Bermudez F Surks M I & Oppenheimer J H J Clin Endocrinol Metab 41 27 1975
3 Bernstein G & Oppenheimer J H J Clin Endocrinol 26 195 1966
4 Braverman L E Vagenakis A Downs P Foster A E Sterling K & Ingbar S H J Clin Invest 52 1010 1973
5 Brown-Grant K Brennan R D & Yates F E J Clin Endocrinol 30 733 1970
6 Burger A Nicod P Suter P Vallotton M B Vagenakis A & Braverman L Lancet I 653 1976
7 Burke C W & Eastman C S Br Med Bull 30 93 1974
8 Carter J N Eastman C J Corcoran J M & Lazarus L Lancet 2 971 1974
9 Chopra I J Ho R S & Lam R J Lab Clin Med 80 729 1972
10 Chopra I J Solomon D H Chopra U Young R T & Teco G N C J Clin Endocrinol Metab 39 501 1974
11 Inada M & Sterling K J Clin Invest 46 1275 1967
12 Larsen P R J Clin Invest 51 1125 1972
13 Laurell C B Anal Biochem 15 45 1966
14 Lauridsen U B Deckert T Friis Th Kirkegaard C Hansen J M & Siersbæk Nielsen K Acta Med Scand 196 171 1974
15 Lutz J H Gregerman R I Spaulding S W Hornick R B & Dawkins A T Jr J Clin Endocrinol Metab 35 230 1972
16 McConnon J Row V V & Volpé R J Clin Endocrinol 34 144 1972
17 Morgan C R & Lazarow A Proc Soc Exp Biol Med 110 29 1962
18 Murphy B P J Lab Clin Med 66 161 1965
19 Nielsen H G Buus O & Weeke B Clin Chim Acta 36 133 1972
20 Nomura S Pittman C S Chambers J B Jr Buck M W & Shimizu T J Clin Invest 9 643 1975
21 Oppenheimer J H Squef R Surks M I & Hauer H J Clin Invest 42 1769 1963
22 Patel Y C & Burger H G J Clin Endocrinol Metab 37 190 1973
23 Pittman C S Chambers J B Jr & Read V H J Clin Invest 50 1187 1971
24 Rubenstein H A Butler V P Jr & Werner S C J Clin Endocrinol Metab 37 247 1973
25 Sterling K & Brenner M A J Clin Invest 45 153 1966
26 Sterling K & Chodos R B J Clin Invest 35 806 1956
27 Surks M I Schadlow A R Stock J M & Oppenheimer J H J Clin Invest 52 805 1973
28 Wintrobe M M Clinical hem ... p 1125 Lea & Febiger Philadelp
29 Woeber K A & Ingbar S H J ... 1968

Acta Med Scand 202 481–487 1977

On the Diagnosis of So-called Normocalcaemic Hyperparathyroidism

Ib Transbøl[1]

From Medical Department F Gentofte Hospital Hellerup Copenhagen Denmark

ABSTRACT With the purpose of evaluating their relative efficiency in detecting hypercalcaemia in hyperparathyroidism (HPT), *simultaneous* determinations have been carried out of serum total calcium (TOCa) in a routine laboratory and serum TOCa, ultrafiltrable (UFCa) and ionized calcium (Ca^{++}) in a research laboratory All 69 patients in this series, except one were hypercalcaemic as judged from Ca^{++} and/or UFCa and the diagnosis was verified operatively in all The prevalence of *normocalcaemic* HPT was 48% as judged from routine TOCa 14% from research TOCa, 7% from UFCa and 0–1% from Ca^{++} Thirty three patients had normocalcaemic HPT as evaluated by the routine TOCa technic, but in as many as 23 hereof (70%) this "normocalcaemia" appeared to be due to inappropriate normal standards or laboratory errors Of the remaining 10 patients (30%), being truly normocalcaemic, two had serum calcium lowering conditions, while eight appeared to have no other explanation for their normocalcaemia than a modest degree of HPT Determinations of UFCa and Ca^{++} were essential in the detection of minimal degrees of hypercalcaemia in nine of the 10 cases of true normocalcaemic HPT and valuable for the establishment of a firm diagnosis in a further 10 cases of borderline hypercalcaemia Percentages of 10–20 and 0 2 respectively, are given as approximate figures for the prevalences of normocalcaemic patients in HPT, and of HPT, normocalcaemic+hypercalcaemic, in adults attending out patient clinics

Ever since Mather (24) described the first case of normocalcaemic hyperparathyroidism (HPT) (the *average* level of serum total calcium being at the upper limit of or within the reference range (mean ±2 S D)) the reality of this condition has been a matter of dispute Evidence for normocalcaemic HPT has been produced by phosphate depletion (1 9 26) by tests supposed to reveal parathyroid autonomy (12 15 28 34 35) or hyperfunction (30) and most directly by the use of accurate technics for the determination of serum ultrafiltrable or ionized calcium (11 18 21 22 23 25 31 32 36) Although normocalcaemic HPT has been reported to make up 13–18% of the patients in series including 60 (32) to 179 cases (15) of HPT other investigators claim that the condition is virtually non existent (7 8 14 19 27) Their opinion is covered by the words of Gordan and Roof (14) and Keating (19) stating that normocalcemic hyperparathyroidism is usually attributable to use of inappropriate normal standards, laboratory error or coexistence of conditions which lower the serum calcium level such as intestinal malabsorption acute pancreatitis nephrosis infarction of a parathyroid adenoma or a high dietary level of phosphorus

In the present paper we evaluate the relative efficiency of four methods of serum calcium determination in the detection of hypercalcaemia in 69 cases of HPT and discuss the possible importance of serum calcium lowering conditions in 20 cases of normocalcaemia and borderline hypercalcaemia

STUDY POPULATION

This series includes all of the 69 patients with HPT studied within a 4½ year period who had *simultaneous* determinations of serum calcium carried out with a routine technic for serum total calcium (TOCa) and a research technic for serum TOCa and ultrafiltrable calcium (UFCa) All but nine patients also had *simultaneous* determinations of

[1] *Present address* Division of Endocrinology Department of Internal Medicine Hvidovre Hospital DK 2650 Hvidovre Copenhagen Denmark

Table 1 *Further information on 20 hyperparathyroid patients being either normocalcaemic (n=10) or borderline hypercalcaemic (n=10) as evaluated by the research technic for total calcium determination*

Pat no initials age (y) sex	Mean TOCa Ul Ca[a] Ca[++] Preop	 Postop	Mean preop proteins[d] phosphate CCr[f]	Parathyroid glands[k]		TOCa lowering conditions
Adenomas or hyperplasia (n=13)						
57[e] FCWJ 47 ♂	10.8 (6) 8.3 7.4	– – –	7.1 2.8 74	25×5 mm	A r	Chronic pancreatitis gastric resection
59 JHM 57 ♂	10.8 (1) 8.2 7.5	– – –	– 3.6 100	35×20×6 mm (3 similar glands removed earlier)	H r	None
67[e] KAEJ 65 ♀	10.8 (3) 7.8 6.9	– – –	6.8 4.0 20	14×10×5 mm 10×5×3 mm	A r N r 2 N b	Renal insufficiency high serum phosphate
79[e] THS 30 ♂	10.8 (1) 7.4 6.8	10.0 7.7 6.7	7.1 4.3 87	43 mg	H r H r N r	Neurofibromatosis high serum phosphate
85[e] OHWH 42 ♂	10.8 (2) 7.6 –	– – –	7.9 3.1 93	Enlarged[l] 60 mg	H r H r	None
21 MH 46 ♂	10.7 (7) 7.9 6.9	10.1 7.3 6.4	7.2 2.7 147	30×3 mm	A r N r	None
60 IWN 76 ♀	10.7 (1) 8.5 7.5	 – 	6.7 2.6 82	30×20×20 mm	A r N r	None
78[e] KAM 55 ♀	10.7 (4) 7.9 7.1	9.5 7.4 6.4	7.2 3.1 113	430 mg	A r 3 N b	None
23 FI 58 ♀	10.6 (1) 7.6 6.7	10.2 7.4 6.6	7.4 2.7 85	8×6 mm	A r N r N r	None
27 ALF 55 ♀	10.5 (3) 8.5 7.3	9.0 7.0 6.7	5.0 4.2 40	10×5 mm 10×5 mm 6×4 mm 6×4 mm	micro A r N r micro A r N b	Chronic glomerulo- nephritis hypoproteinaemia high serum phosphate
28 FJ 59 ♂	10.4 (3) 7.7 6.9	9.9 7.3 6.5	6.5 2.7 93	10×7 mm	A r N r	None
77[e] FCMH 44 ♀	10.4 (3) 8.0 6.8	– – –	6.5 3.4 74	370 mg 55 mg 30 mg	A r N r N r	None
20[e] NKC 62 ♀	10.3 (2) 7.8 7.0	10.0 7.2 6.4	7.0 3.2 85	10×10 mm (1 A removed earlier)	A r	Gastric resection biochemical osteo- malacia 1 year after operation
"Normal" parathyroid glands (n=7)						
80[e] EW 51 ♂	10.8 (3) 8.4 7.9	9.9 7.5 –	7.3 4.6 13	15×10×8 mm 8×6×3 mm 5×5×3 mm	N r N b N b N r	Renal insufficiency high serum phosphate high phosphate intake
25 HSM 30 ♂	10.7 (1) 7.8 6.9	9.8 6.8 6.2	7.3 3.3 73	22×4 mm 8×4 mm	N r N r	None

Table I (continued)

Pat no initials age (y) sex	Mean TOCa ᵃ UFCa ᵇ Ca ⁺⁺ᶜ		Mean preop proteins ᵈ phosphate CCr ᶠ	Parathyroid glands ᵏ		TOCa lowering conditions
	Preop	Postop				
27	10 6 (3)	9 6	6 9		N r	None
MVH	8 4	7 4	3 2			
61 ♀	7 7	6 4	60			
76	10 4 (3)	9 9	7 5	8×3×2 mm	N r	None
PVN	7 8	7 2	2 5	*8×3×2 mm*	*N r*	
43 ♂	7 0	6 4	129	8×3×2 mm	N r	
29	10 4 (3)	9 8	7 9	5×5 mm	N r	None
ALJ	8 0	6 8	3 5	5×5 mm	N r	
53 ♂	7 0	–	82			
30	10 2 (7)	9 9	6 9	5×3 mm	N r	None
VPS	7 8	7 2	3 3			
45 ♂	6 9	6 4	113			
61	9 8 (3)	9 6	7 0	5×3×2 mm	N r	None
HH	7 5	7 2	3 3	3×3×3 mm	N r	
77 ♂	6 7	6 2	50			

ᵃ Serum total calcium ᵇ ultrafiltrable calcium ionized calcium (mg/100 ml) determined by research technics Values within parentheses indicate the number of observations from which each set of figures is derived ᵈ Serum proteins (g/100 ml) serum phosphate (mg/100 ml) ᶠ 24-hour creatinine clearance (ml/min) ᵍ Studied on diets having a calculated or measured phosphorus content averaging 1 133 mg/24 h (range 838–1 433) see Transbøl et al (33) Table I for separate data ʰ High phosphorus intake (2 416 mg/24 h) ⁱ Low calcium low phosphate diet ᵏ A=adenoma N=normal H=hyperplasia r=removed b=biopsy

serum ionized calcium (Ca^{++}) with a research technic The series includes 44 patients from a previous paper (32) leaving out 16 patients in whom routine determinations were not done and adding 25 new patients According to our procedure all patients suspected of hypercalcaemia undergo an initial evaluation with routine TOCa determinations those with values above or within the upper half of the reference range then proceed to the simultaneous determinations Since nearly all patients presented with symptoms compatible with a diagnosis of HPT operation was recommended when 1) the average Ca^{++} concentration (60 patients) or UFCa concentration (8 patients) was at or above the 99% confidence limit and 2) no other cause of hypercalcaemia could be demonstrated One patient (no 85) did not satisfy the first criterion He had the unique combination of a slightly elevated TOCa a high normal UFCa and no Ca^{++} determinations

The diagnosis was confirmed by light microscopic demonstration of adenoma(s) or hyperplasia (60 cases) and/or a sustained normalization of Ca initiated by the neck exploration (9 cases) (32) As judged from the research TOCa method 20 patients were normocalcaemic or had borderline hypercalcaemia (average TOCa at or within the 99% confidence limit) Further details on the pre per and postoperative evaluation and the presence of TOCa lowering conditions in these patients are presented in Table I

METHODS

Blood was drawn in fasting state in the sitting position at 7–9 a m Nearly all patients walked to the laboratory A tourniquet and wide gauged needles were used For the first 4 years the routine determinations of TOCa were carried out by the titremetric method of Bett and Fraser (2) with a reference range of 8 3–10 8 mg/100 ml (mean ±2 S D) Fifty nine patients were studied by this method and the other 10 by atomic absorption spectroscopy (model I L 153) reference range 9 2–10 8 mg/100 ml These 10 patients included seven of those in whom Ca^{++} determinations were omitted TOCa UFCa and Ca^{++} were determined in the research laboratory by a modification of Rose s method (32) Routine technics referred to previously (31 32) were used for determinations of serum proteins and phosphate and serum and urinary creatinine The reference ranges for proteins and phosphate were 6 3–8 3 g/100 ml and 2 5–4 6 mg/100 ml respectively

RESULTS

Average values for routine TOCa and TOCa UFCa and Ca^{++} determined by research methods for each of the 69 patients are presented in Fig 1 The various correlations between research TOCa UFCa and Ca^{++} are outlined in Fig 2 The degree of correlation between routine TOCa and Ca^{++} research TOCa and UFCa research TOCa and Ca^{++} and UFCa and Ca^{++} is characterized by Spearman s coefficient of rank correlation R measuring 0 86 0 90 0 89 and 0 93 respectively The excellent cor-

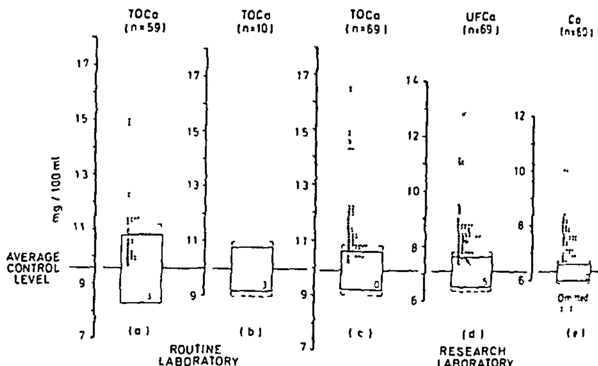


Fig. 1. Average concentrations of routine serum total calcium determinations (*TOCa* (*a*) and (*b*)) and of determinations performed in the research laboratory of TOCa (*c*) ultrafiltrable calcium (*UFCa* (*d*)) and ionized calcium (*Ca* (*e*)) in 69 patients with hyperparathyroidism. The 95 and 99% ( ) confidence limits are indicated for each technic. The figures within the reference areas indicate the number of patients who were classified as normocalcaemic by the technic in question. The arrows in column (*d*) point to the concentrations of UFCa in the nine patients in whom Ca determinations were omitted.

lation between UFCa and Ca^{++} (Fig. 2*c*) and the finding of rather high values of UFCa in eight out of the nine patients without Ca^{++} determinations (arrows in Fig. 1*d*) indicate almost certainly that these eight patients also had raised levels of Ca^{++}.

As judged from the routine TOCa determinations, 33 patients (48%) were normocalcaemic while this fraction was reduced successively to 14, 7 and 0–1% when the research methods for TOCa, UFCa and Ca^{++} were considered (Fig. 1 and Table II).

Additional data on 20 patients who were either normocalcaemic ($n=10$) or borderline hypercalcaemic ($n=10$) as evaluated by the research TOCa method (Fig. 1*c*) are presented in Table I. It appears that one or more of the generally accepted TOCa lowering factors such as conditions favouring malabsorption, high serum phosphate levels, high intake of phosphate and hypoproteinaemia were present in six out of 20 cases (2 normocalcaemic and 4 borderline). In the remainder, serum proteins were unmeasured in one and within the reference range in 13 cases. In eight normocalcaemic and five borderline cases the serum proteins averaged 7.1 and 7.3 g/100 ml respectively. The deviation of the normocalcaemic patients with respect to serum proteins averages −0.2 g/100 ml corresponding to about −0.1 mg/100 ml TOCa.

In 13 of these 20 patients operations revealed parathyroid abnormalities satisfying the conventional criteria for hyperplasia or adenomas (Table I). Parathyroid glands which appeared suspicious to the surgeon were removed in the remaining seven patients. Although some of these glands were obviously enlarged, all were judged to be normal by the pathologist (Table I).

Patients with adenomas or hyperplasia ($n=13$) had average levels of research TOCa, UFCa and Ca^{++} of 10.64, 7.94 and 7.07 mg/100 ml respectively, not differing significantly from the respective values of 10.41, 7.96 and 7.16 mg/100 ml in patients harbouring "normal" glands ($n=7$). Seven patients from each group also had postoperative de[illegible]

Table II *Frequency of normocalcaemic hyperparathyroidism as a measure of the relative efficiency of routine and research methods in detecting hypercalcaemia in 69 cases of hyperparathyroidism*

	Reference range (mg/100 ml) Mean ±2 S D	Width	Normocalcaemic HPT (%)
...tine TOCa (a)	8 3–10 8	2 5	51
...tine TOCa (b)	9 2–10 8	1 6	30
...tine TOCa (a+b)			48
...earch TOCa (c)	9 2–10 6	1 4	14
...earch UFCa (d)	6 6– 7 7	1 1	7
...earch Ca⁺⁺ (e)	6 0– 6 6	0 6	0–1

...rathyroidectomy was not recommended unless the ...age concentration of Ca^{++} was 6 7 mg/100 ml or ...ve

...ons carried out by the research methods usu... more than three weeks after the operation ...ble III) The average pre and postoperative ...es were identical for TOCa and UFCa as well ...for Ca^{++}

DISCUSSION

...cause of unequal working conditions it is not fair ... compare results derived from routine and re...arch technics However such comparisons may ...ve important practical implications It is im...rtant to clinicians and to clinical chemists to ...ow that some 48% of patients suffering from ...PT appear to be normocalcaemic—and therefore ...main undiagnosed—when evaluated by routine ...OCa determinations This proportion can be re...ced from about 48 to 14% simply by improving ... conditions under which the determinations of ...OCa are carried out Thus the main cause of

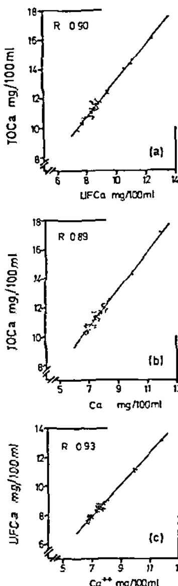


Fig 2 Correlations between simultaneous determinations of serum total (*TOCa*) ultrafiltrable (*UFCa*) and ionized calcium (*Ca⁺⁺*) carried out by research technics R = Spearman s coefficient of rank correlation

...able III *Relation between parathyroid pathology and the response to parathyroidectomy in hyperpara...yroid patients being either normocalcaemic or borderline hypercalcaemic*

	No of subj	TOCa (mg/100 ml) Preop	Postop	UFCa (mg/100 ml) Preop	Postop	Ca⁺⁺ (mg/100 ml) Preop	Postop
...denoma or hyperplasia	7	10 57	9 81	7 83	7 33	6 96	6 46
...Normal" glands	7	10 41	9 79	7 96	7 16	7 16	6 32 (n=9)
...eference range	81	9 2–10 6		6 55–7 65		6 00–6 60	

apparently normocalcaemic HPT appears to be the use of inappropriate normal standards or laboratory errors as suggested by Gordan and Roof (14) and Keating (19) Like Davies et al (8) we feel that it is better to spend efforts on doing serum calcium determinations very accurately than on performing various tests like phosphate depletion calcium infusion etc

In a previous paper (32) we reviewed the literature on determinations of UFCa and Ca^{++} and presented considerable evidence indicating that such determinations may detect minimal hypercalcaemia in hyperparathyroid patients judged to be normocalcaemic by an accurate TOCa technic Davies et al (8) have had similar experiences with another modification of Rose s method and more recent studies mainly based on the Ca^{++} electrode also confirm our observations (11 18 21 23 25)

TOCa *lowering conditions* claimed by Gordan and Roof (14) and Keating (19) to be *responsible for* normocalcaemia in HPT were present in four out of ten borderline and two out of ten normocalcaemic cases (Fig 1c Table I) Since serum proteins averaged 7 3 and 7 1 g/100 ml respectively in the remaining cases of borderline and normocalcaemic HPT nothing but a modest degree of HPT appears to be responsible in these 14 cases Regardless of the presence of TOCa lowering conditions it is important to make a firm diagnosis of rcalcaemia and we therefore consider the de inations of UFCa and Ca to have been of at diagnostic importance in all but one (no 85) of hese 20 cases On the other hand we have reasons to believe that some normocalcaemic patients have remained undiagnosed These reasons are firstly that we used the routine TOCa technic for the purpose of initial evaluation and secondly that we did not recommend operation unless the average UFCa and/or Ca concentration exceeded the 99% confidence limit Since this limit is 6 7 mg/100 ml for Ca^{+} and the concentration hereof declines 0 5 mg/100 ml on an average following parathyroidectomy in the borderline group (Table III) patients who have had low normal Ca concentrations ahead of their disease may supposedly develop hypercalcaemia of clinical significance—and remain undertected

The detection over the last 10 years of an increasing number of patients with borderline or normocalcaemic HPT (15 25 28 30 32 34) has created new problems for the surgeon and for the pathologist as well As the routine TOCa techn has shortcomings so do routine technics in surgery and pathology The observations presented in Table I are illustrative Normal size borderline and slightly enlarged glands measuring 43 mg 60 mg and 8×6 mm may be hyperplastic (patients 79 and 8) or adenomatous (patient 23) while huge glands measuring 15×10×8 mm and 22×4 mm may have a normal histological appearance (patients 80 and 25) This discrepancy even occurred within one and the same patient (no 27) who had a modestly enlarged gland (6×4 mm) containing several microadenomas as well as a 10×5 mm gland which appeared normal to the pathologist Regardless of the pathology patients had similar degrees of hypercalcaemia and responded similarly to parathyroidectomy (Table III) At first we were nonplussed but as the same happened over and over again we were *forced to* believe in the reality of these observations (3) Fortunately others have had similar experiences initially Bartter s (12) and Frame s groups (4) followed by other groups in the recent years (1 13 17 23 30) It has now been established that glands which appear normal to the naked eye and even in the light microscope may reveal distinct electron microscopic signs of secretory hyperactivity (3 30) Unfortunately this does not solve the problems posed at the operating table and resection of 3–3½ glands has therefore become the *operation of choice in many* patients with borderline or normocalcaemic HPT not showing evident macroscopic or light microscopic abnormalities (4 10 16 29 30) This makes a simple necessity of being able to identify *all* parathyroid glands with a minimum risk of damaging the recurrent nerves or causing lasting hypoparathyroidism In the hands of the experienced surgeon a 3–3½ gland resection does not carry an appreciable risk of these complications (10 16 29 30)

One of the most important *observations* in the present paper seems to be the fact that the routine TOCa technic at a university hospital may leave nearly 50% of hyperparathyroid patients undiagnosed This has an important bearing on studies on the prevalence of HPT in the adult population Since such studies based on observations in outpatient clinics reveal a prevalence of *hyper-calcaemic* HPT of about 1 1000 as detected by routine TOCa technics (5 6) and 1 700 by a very accurate TOCa technic (20) the present observation suggests that the prevalence of HPT

normocalcaemic plus hypercalcaemic might be higher even 1 500

Let us finally return to the deviating viewpoints presented in the preface This study confirms the cited suggestions by Gordan and Roof (14) and Keating (19) in two respects Firstly it documents that most of the patients characterized as nor mocalcaemic by a routine TOCa technic can be diagnosed as being hypercalcaemic by a more accu rate technic and secondly it shows that maybe 70–40% of those found to be truly normocalcaemic are afflicted with TOCa lowering conditions which conceal a more severe degree of HPT However instead of detracting from the reality of the normocalcaemia these conditions just make a proper diagnosis more important Finally this study suggests that series of hyperparathyroid patients which include less than 10–20% of normocalcaemic cases leave a significant number of patients un diagnosed

REFERENCES

1 Adams P Chalmers T M Hill L P & Truscott B M Br Med J 4 582 1970
2 Bett I M & Fraser G P Clin Chim Acta 4 346 1959
3 Black W C & Haff R C Am J Clin Pathol 53 565 1970
4 Block M A Greenawald K Horn Jr R C & Frame B Am J Surg 114 530 1967
5 Boonstra C E & Jackson C E Ann Intern Med 63 468 1965
6 — Am J Clin Pathol 55 523 1971
7 Cope O & Goldman L JAMA 228 1038 1974
8 Davies D R Dent C E & Watson L Br Med J 1 108 1971
9 Eisenberg E J Clin Endocrinol 28 651 1968
10 Esselstyn Jr C B Levin H S Eversman J J Schumacher O P & Skillern P G Surg Clin North Am 54 443 1974
11 Geis H P Popovtzer M M Corman J L Halgrimson C G Groth C G & Starzl T E Surg Gynecol Obstet 137 997 1973
12 George J M Babson A S Ketcham A & Bartter F C Q J Med 34 291 1965
13 Goldsmith R E Gall E A Altemeier W A Weinsten A & Zalme E Ann Intern Med 75 395 1971
14 Gordan G S & Roof B S JAMA 206 2729 1968
15 Grimelius L Johansson H Lindquist B Thoren L & Werner I Acta Chir Scand 139 42 1973
16 Haff R C & Armstrong R G Surgery 75 715 1974
17 Irvin III G L Cohen M S Moebus R & Mintz D H Arch Surg 105 738 1972
18 Jørgensen F S Transbøl I & Binder C Acta Med Scand 194 323 1973
19 Keating Jr F R Med Clin North Am 54 511 1970
20 Keating Jr F R Jones J D & Elveback L R J Lab Clin Med 49 507 1969
21 Ladenson J H & Bowers Jr G N Clin Chem 19 575 1973
22 Lloyd H M & Rose G A Lancet 2 1258 1958
23 Low J C Schaaf M Earll J M Piechocki J T & Li T K JAMA 223 152 1973
24 Mather H G Lancet 2 424 1953
25 Muldowney F P Freaney R Spillane E A & O Donahoe P Ir J Med Sci 142 223 1973
26 Nichols Jr G & Flanagan B Trans Assoc Am Physicians 80 314 1967
27 Nordin B E C Metabolic bone and stone disease p 105 Churchill Livingstone Edinburgh and London 1973
28 Pak C Y C East D Sanzenbacher L Ruskin B & Cox J Arch Intern Med 129 48 1972
29 Paloyan E Lawrence A M Baker W H & Straus II F H Surg Clin North Am 49 43 1969
30 Shieber W Birge S J Avioli L V & Teitelbaum S L Arch Surg 103 299 1971
31 Transbøl I Hahnemann S & Hornum I Acta Med Scand 184 33 1968
32 — Acta Endocrinol (Kbh) 65 385 1970
33 Transbøl I Hornum I Hahnemann S Hasner E Øhlenschlæger H Diemer H & Lockwood K Acta Med Scand 188 505 1970
34 Wibell L Johansson H & Werner I Scand J Clin Lab Invest 30 183 1972
35 Wills M R Pak C Y C Hammond W G & Bartter F C Am J Med 47 384 1969
36 Yendt E R & Gagne R J A Can Med Assoc J 98 331 1968

Acta Med Scand 202 489–494 1977

Treatment of Glomerulonephritis with Drainage of the Thoracic Duct and Plasmapheresis

Uffe Ravnskov Olle Dahlbäck and Lisbeth Messeter

From the Departments of Nephrology and Thoracic Surgery and the Blood Bank University Hospital Lund Sweden

ABSTRACT Nine patients with various types of severe glomerulonephritis were treated with drainage of the thoracic duct ($n=8$) and/or plasmapheresis ($n=6$) without the use of pharmacological immunosuppression In most of the patients treatment produced a prompt temporary regression of albuminuria and creatininemia In one patient renal function was substantially improved 8 months after the last period of treatment in 2 patients the progress of the uremia was probably retarded In the remaining 6 patients treatment had no obvious effect on the course of the illness Drainage of the thoracic duct and plasmapheresis may be of benefit in the treatment of glomerulonphritis, but their proper utilization requires further studies

Much evidence speaks in favour of circulating nephrotoxic macromolecules (immune complexes or anti GBM antibodies) as mediators at least partly of the renal damage in SLE nephritis Goodpasture s syndrome and possibly also in other forms of progressive glomerulonephritis Attempts to decrease the synthesis of immunoglobulins by drugs have been tried with some success in SLE and Wegener s granulomatosis but have usually failed in non systemic glomerulonephritis Elimination of possible pathogenic substances or cells by drainage of the thoracic duct has had a temporary effect in rheumatoid arthritis (10) SLE arthritis (7) myasthenia gravis (1) and after renal transplantation (3) and has also been tried in glomerulonephritis (ref 1a) Treatment with plasmapheresis combined with pharmacologic immunosuppression has been reported to be beneficial sometimes in Good pasture s syndrome (4 5) in SLE nephritis (9) and in extracapillary glomerulonephritis ((

In view of the gloomy prognosi of progressive glomerulonephritis it was thought legitimate to try drainage of the thoracic duct and/or plasmapheresis without the use of chemical immunosuppression in the treatment of different types of progressive glomerulonephritis

PATIENTS AND METHODS

The clinical material is summarized in Table I During and on the few days after the end of the treatment the following determinations were made every day serum creatinine the albumin/creatinine clearance (A/C) ratio (8) serum albumin IgG IgA orosomucoid fibrinogen WBC and differential count C3 and C4 Before treatment was started plasma and lymph were examined for anti GBM antibodies (by courtesy of Ph Mahieu Liège)

Plasmapheresis was done manually and with disposable plastic equipment About 800 ml of plasma was removed on each occasion The thoracic duct was drained via an indwelling polyethylene catheter

In patients who received both kinds of treatment treatment was started with lymph drainage followed by plasmapheresis 2–4 weeks after the end of the drainage period On days of treatment the patients were given 200–300 ml stored human plasma

RESULTS

Drainage of the thoracic duct was followed by a fall of the mean serum creatinine level to 84% of the value measured immediately before the treatment One of the patients developed oliguria owing to insufficient intake of salt and water during the treatment period During treatment his serum creatinine level rose from 260 to 470 μmol/l but returned to the original level after rehydration When this patient is excluded the mean serum creatinine after treatment was 71% (range 40–

ig 1 Table I)

Table I *Pertinent clinical data of 9 patients treated with drainage of the thoracic duct (LD) and or pl[...] pheresis (PP)*

All patients except nos 2 and 4 had been previously treated with prednisolone and azathioprine without benef[...] Pa[...] and 9 received 10 mg prednisolone daily during treatment
GLN = glomerulonephritis A/C ratio = albumin/creatinine clearance ratio

Pat no age (y) sex	History and findings	Date of renal biopsy and findings	LD period (d)	Treatments	Δ Serum crea[...] (μmol/l)
1 36 ♂	Nephrotic syndrome in Aug 1975 Exposed to organic solvents	Jan 1976 Membranoproliferative GLN	11	LD 1st PP 2nd PP 3rd PP 4th PP	[illegible] [illegible] [illegible] [illegible] [illegible]
2 65 ♂	Painter Proteinuria demonstrated in 1943 Pneumonia ventricular ulcer and nephrotic syndrome in Nov 1975	Feb 1976 Membranoproliferative GLN	10	LD 1st PP 2nd PP	[illegible] [illegible] [illegible]
3 30 ♀	SLE arthritis nephrotic syndrome in 1969 Progressive renal failure since Oct 1975	1969 Proliferative GLN	14	LD 1st PP 2nd PP	[illegible] [illegible] 1 050-1[...]
4 59 ♀	For 15 years mild hypertension urinary tract infections asthmatic bronchitis Raised serum creatinine discovered in Jan 1976 No evidence of polyarteritis	March 1976 Extracapillary GLN crescents in 6 of 10 glomeruli		1st PP 2nd PP	[illegible] [illegible]
5 42 ♂	Proteinuria found accidentally in 1973	March 1974 Focal segmental proliferative GLN	5	LD 1st PP	210-14[...] [illegible]
6 65 ♂	Nephrotic syndrome in 1975 Asthma during 1976 eosinophilia no evidence of polyarteritis	Feb 1975 Lobular proliferative GLN	8	LD 1st PP	[illegible] [illegible]
[illegible]	SLE arthritis in Feb 1974 Acute nephritis in June 1975	Nov 1975 Lobular proliferative GLN	5	LD	[illegible]
8 38 ♂	Painter Ulcerous colitis in 1962–66 Proteinuria found accidentally in 1969	1970 Lobular proliferative GLN	7	LD	[illegible]
9 17 ♂	Probably poststreptococcal GLN in April 1976 In the following months intestinal and pulmonary bleedings Exposed to organic solvents	Aug 1976 Extracapillary GLN with crescents in 6 of 8 glomeruli	12	LD	[illegible]

* Developed oliguria during treatment owing to insufficient intake of salt and water

Proteinuria measured as the A/C ratio decreased after lymph drainage to 33 5% (range 12–87) of the original value (Fig 2) Measured as the mean albumin concentration in a morning urine sample it decreased to 28 6% (range 13–46) of that noted before treatment The mean serum albumin concentration after the treatment was 100 5% (range 79–150) of the pretreatment value (Table I)

Plasmapheresis was followed by a decrease in the mean serum creatinine to 84 3% (range 53–105) of the original value in the mean A/C ratio to 56 1% (range 23–113) and in the mean albumin concentration in a morning urine sample to 64 1% (range 40–103) The mean serum albumin increased to 107% (range 71–129) of the original value (Figs 1 2 Table I)

The changes in the serum creatinine levels appeared mostly after a few days treatment In [...]

Δ A/C ratio 10⁹	Δ Morning urinary albumin (mg/l)	Lymph or plasma removed (l)	Follow up
23 6-3 7	17 700-2 500	15 9	Serum creatinine 810 μmol/l in Jan 1977 3 months after the latest PP
14-8 2	8 950-7 750	2 4	
13 1-7 5	12 450-8 050	1 6	
14 8-5 5	11 400-6 500	3 3	
21 4-11 9	8 000-4 500	6 4	
13 5-2 6	1 250-575	11 8	Serum creatinine 770 μmol/l in Oct 1976 3 months after the latest PP
10 3-9 7	1 550-1 600	2 7	
5 4-6 1	1 330-1 200	3 7	
11 1-4 2	900-330	33 5	On dialysis treatment since Oct 1976
17-4 4	1 300-650	6 5	
76-75	3 700-2 300	5 7	
2 3-0 64	785-120	5 9	Serum creatinine 250 μmol/l and A/C ratio 0 63 10⁻³ in Oct 1976 6 months after the latest PP
1 25-1 71	1 000-400	4 0	
1 84-0 61	2 000-490	5 2	Died 2 months after the latest PP in May 1976 in ventricular cancer
1 07-0 46	1 000-700	5 4	
10 1-8 8	2 750-1 250	12 1	Died in Nov 1976 in renal and respiratory failure 4 months after the latest PP
14 7-5 3	4 100-1 950	5 3	
5 41-1 97	8 750-2 550	13 4	Serum creatinine 75 μmol/l 5 months after LD
1 14-0 35	1 500-220	14	On dialysis treatment since June 1976
8 2-0 97	3 900-500	36	On dialysis treatment 2 months after LD

patients the decrease continued for up to 10 days after the beginning of the treatment (Figs 3 4 5) in one patient the decrease started after the end of drainage of the thoracic duct (Fig 4) In one patient the effect on serum creatinine lasted 8 months after plasmapheresis (Fig 4) in 2 patients the creatinine gradually increased during 3 months and in the others serum creatinine remained unchanged during treatment or increased almost immediately after treatment to the original or an even higher level

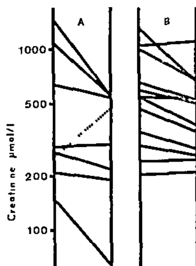


Fig 1 Serum creatinine (log scale) before and after drainage of the thoracic duct (*A*) and plasmapheresis (*B*) ---=patient 1 who developed prerenal oliguria during treatment

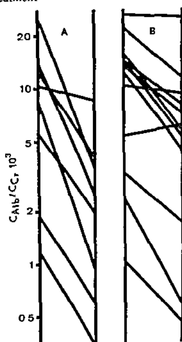


Fig 2 Albumin/creatinine clearance ratio (log scale) before and after drainage of the thoracic duct (*A*) and plasmapheresis (*B*)

The excretion of albumin decreased promptly (1-3 days) after the beginning of the treatment and increased immediately after the end of the treatment (Figs 5 6) in most patients only to a lower level than that noted before treatment

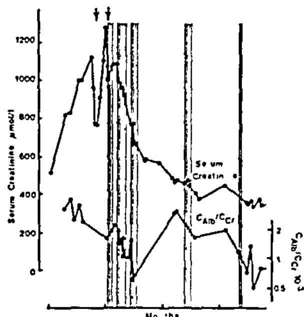


Fig 3 Course in patient 4 (extracapillary glomerulonephritis) Vertical line denotes pheresis of about 800 ml plasma arrow = hemodialysis At the latest follow-up 8 months after the latest plasmapheresis serum creatinine was 250 µmol/l

No significant variations were observed in the plasma proteins complement components or WBC

DISCUSSION

[C]aution must be excercised in the evaluation of any [unc]ontrolled study especially when the number of [pa]tients is small as in this investigation Yet in our patients plasmapheresis and drainage of the thoracic duct did appear to influence the course of the disease though usually for only a short time since the changes in proteinuria and serum creatinine occurred promptly after the beginning of treatment and disappeared soon after treatment had stopped

The mechanism underlying this temporary improvement in renal function is obscure In contrast to other studies using plasmapheresis the effect could not be explained by the simultaneous use of immunosuppressive drugs The only such drug was prednisolone given to 2 patients only in small doses which were reduced before and during the treatment

Plasmapheresis has been claimed to have a beneficial effect by its elimination of immune complexes anti-GBM antibodies complement components and fibrinogen (5 6) but in none of our patients could circulating anti-GBM antibodies be detected in the plasma or in the lymph and no significant changes were demonstrable in C3 C4 or fibrinogen We were not able to measure circulating immune complexes and can therefore not exclude the possible elimination of nephrotoxic immune complexes Circulating complexes have rarely been demonstrated in glomerulonephritis except in SLE In the 2 patients with SLE treated by us the C3 and C4 levels were normal at the time of the treatment and thereby argued against the presence of circulating complexes

Depletion of lymphocytes by lymph drainage or non specific temporary restoration of immune competence of T lymphocytes following plasmapheresis (9) are possible causes though most workers believe that cell mediated immunity is of little or no importance in the causation of chronic glomerulonephritis

The value of thoracic duct drainage in the treatment of glomerulonephritis may seem limited since for technical reasons such treatment can be given only once and for a short period However since the generation of nephritogenic substances may be self limiting (5 6) lymph drainage may be of value in selected cases alone or combined with plasma-

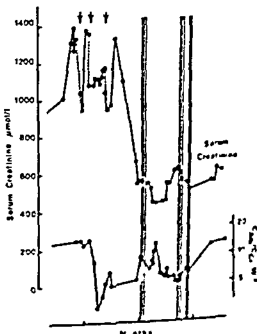


Fig 4 Course in patient 2 (membranoproliferative glomerulonephritis) ▨ = period of drainage of thoracic duct arrows = peritoneal dialysis vertical lines as in Fig 3

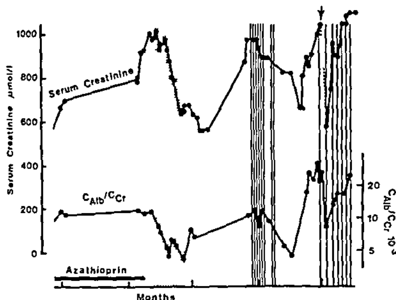


Fig 5 Course in patient 3 (SLE nephritis) Symbols as in Figs 3 and 4

pheresis Furthermore plasmapheresis is more laborious and expensive than thoracic duct drainage Thus even though such drainage cannot be repeated it may increase the therapeutic potential

The excellent result we obtained in the first patient (no 6) treated with plasmapheresis induced us to omit the use of immunosuppressive drugs in the other patients too This may explain why treatment usually had only a temporary effect Drainage of the thoracic duct combined with a more frequent and effective plasmapheresis such as can be achieved with a continuous flow blood cell separator and chemical immunosuppression may perhaps give better long term results

In the evaluation of our results it might not be out of place to mention that at the time of treatment 3 of our 4 patients were in a slowly progressive stage of the disease and that at least 4 of the other 6 patients had been slowly deteriorating for 6 months to 8 years before the rapidly progressive course during which they were treated The treatment should perhaps be reserved for patients who have not had the illness for such a long time and in whom the inflammatory changes of the kidney have not become irreversible

Future research should probably be focused on the unknown factors whose elimination is followed by the improvement of renal function Knowledge of such factors would presumably be useful in the selection of patients most likely to respond to these new kinds of treatment

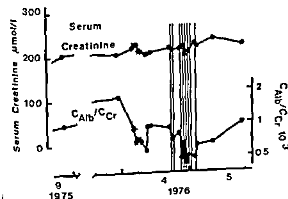


Fig 6 Course in patient 5 (lobular proliferative glomerulonephritis) Symbols as in Figs 3 and 4

REFERENCES

1 Bergstrom K Franksson C Matell G & von Reis G The effect of thoracic duct lymph drainage in myasthenia gravis Eur Neurol 9 157 1973

1a Bonomini V Mioli V Albertazzi A & Vangelista A Immunosuppressive drugs and lymphatic depletion by thoracic duct fistula in adult progressive glomerulonephritis Nephron 7 389 1970

2 Browne O Bell J Holland P D J & Thornes R D Plasmapheresis and immunostimulation Lancet 2 96 1976

3 Franksson C Drainage of the thoracic lymph duct during homologous kidney transplantation in man In Kidney transplantation (ed C Franksson) p 51 Almqvist & Wiksell Uppsala 1968

4 Lockwood C M Boulton Jones J M Lo R M Simpson I J Peters D K &

Recovery from Goodpasture's syndrome after immunosuppressive treatment and plasmapheresis. Br Med J 2: 252, 1975

5. Lockwood C M, Pearson T A, Rees A J, Evans D J, Peters D K & Wilson C B. Immunosuppression and plasma-exchange in the treatment of Goodpasture's syndrome. Lancet 1: 711, 1976
6. Lockwood C M, Pinching A J, Sweny P, Rees A J, Pussell B, Uff J & Peters D K. Plasma exchange and immunosuppression in the treatment of fulminating immune-complex crescentic nephritis. Lancet 1: 63, 1977
7. Olhagen B & Franksson C. Immediate effects of lymph drainage in systemic lupus erythematosus. p. 265. Abstr. XI Internat Congr Rheumat, Mar del Plata 1965
8. Schreiner G F. The nephrotic syndrome. In: Diseases of the kidney (ed. M B Strauss & L G W...), p. 518. Little Brown & Co, Boston 1971
9. Verrier Jones J, Bucknall R C, Cumming R H, Asplin C M, Fraser I D, Bothamley J, Davis P & Hamblin T J. Plasmapheresis in the management of acute systemic lupus erythematosus? Lancet 1: 709, 1976
10. Wegelius O, Laine V, Lindström B & Klockars M. Fistula of the thoracic duct as immunosuppressive treatment in rheumatoid arthritis. Acta Med Scand 187: 539, 1970

Acta Med Scand 202 495–500 1977

Transient Changes of Sensory Nerve Functions in Uraemia

A H Lang and J Forsström

From the Departments of Clinical Neurophysiology and Medicine University Central Hospital of Turku Turku Finland

ABSTRACT The neurophysiological parameters (conduction velocity, amplitude and rise time of the compound action potential) of two sensory nerves of the lower limb (N suralis and peronaeus superficialis) were determined bilaterally in five patients Measurements were carried out at least twice in each subject during a time of great metabolic change Two patients were recovering from acute renal failure (a single haemodialysis had to be performed on one of them) and three were under chronic maintenance haemodialysis In the case of the haemodialysis patients, measurements were performed on two successive days, before and after one haemodialysis session All the neurophysiological parameters showed significant improvement in successive measurements In one patient the follow up after acute renal failure revealed a delayed deterioration of the nerve functions but after one year the values were normal From the results it seems probable that an acute metabolic disturbance may cause transient inhibition of nerve functions without morphological changes in the nerve fibres

Polyneuropathy in patients with renal failure has been of special interest ever since it became possible to treat chronic uraemia by means of maintenance heamodialysis and kidney transplant Neuropathy as part of the terminal picture of chronic advanced renal failure was first recognized and confirmed pathologically at necropsy by Asbury et al (2) As an electrophysiological criterion and for following dialysis treatment it has been customary to use the electrical functions of the peripheral nerves particularly their maximal motor conduction velocity (NCV) In recent years sensory NCV measurements have also become common The survey by Nielsen (19) contains extensive references to the literature on the subject and a summary of his own systematic observation

It can be noted that Preswick and Jeremy (21) had already ascertained that in cases of chronic renal insufficiency the NCV was sometimes slowed down without there being any certain symptoms or signs of polyneuropathy this observation has been confirmed on numerous occasions (10 11 13 17) Nevertheless the common consensus has been that a slow nerve conduction velocity in chronic uraemia is caused by at least a subclinical polyneuropathy i e it is always associated with morphological changes in peripheral nerve fibres (1)

Most histopathological studies of peripheral nerves in cases of uraemia have as Nielsen (18) points out been mainly concerned with chronic uraemia patients at the terminal stage with manifest polyneuropathy or have been made at necropsy According to Thomas (24) it is questionable whether the slight morphological changes in peripheral nerves can account for the mild slowing of nerve conduction so often observed in initial stages of uraemia Nielsen (17 18) has advanced the theory that the slowing of nerve conduction in chronic uraemia can also be due to an inhibition of the transmembrane sodium pump caused by humoral toxic factor(s) in the serum The slowing down of NCV would thus be functional and reversible in character

If the slowing down of NCV in chronic uraemia was invariably a sign of morphological change in the peripheral nerves it would naturally—in the treatment of uraemia and dialysis patients—have to be taken more seriously than if the same phenomenon in early stages of uraemia and small variations d

Table I *Initial neurophysiological data (1st) and changes on the second day of measurement in cases 2-5 the day following dialysis session and serum level of metabolites*

In case 2 no compound action potential could be recorded initially in N peronaeus dx only

Pat. no and diagnosis	Nerve	Neurophysiological data NCV/(m/sec) 1st	Change	Amplitude of NAP (μV) 1st	Change	Rise time (μsec) 1st	Change
1	Per dx.	42.5	+7 0	10 0	− 0 4	600	–
Acute anuria, not	Per sin	44 6	+5.2	8 0	0	750	+ 50
dialysed	Sur dx.	47 0	+7.2	18 0	− 3.2	700	−150
	Sur sin	46 6	+9 6	11 0	+ 6 8	650	+150
2	Per dx.	–	–	–	–	–	–
Acute anuria	Per sin.	29 9	+1 9	2.5	+ 1 9	250	+350
dialysed	Sur dx.	34 6	+5 8	3 4	+ 3 5	750	+250
	Sur sin	33 7	+8 1	1 8	− 1 1	350	+300
3	Per dx.	41 6	+2 0	6 8	− 0 4	700	+ 50
Chronic uraemia	Per sin.	44 1	+0 1	6 4	+ 0 4	800	− 50
	Sur dx	43.2	+4 4	8 8	+11 1	600	+150
	Sur sin	45 1	+0 7	9 4	+ 4.4	750	−150
4	Per dx.	46.2	−2.1	7 9	+ 1 7	1 000	−150
Chronic uraemia	Per sin	42.2	−0 2	8 6	+ 3 1	550	+200
	Sur dx.	42.7	+5 0	16 7	+11.5	700	+200
	Sur sin	43 9	+2.0	16 6	+ 5 5	700	+150
5	Per dx.	34.2	+2.4	2.3	+ 1.5	650	+200
Chronic uraemia	Per sin	34 8	−1 7	4 0	+ 1 7	850	−200
	Sur dx	39 1	+0 4	10 2	− 1 3	1 000	− 50
	Sur sin	38 0	−0.2	7 9	+ 1 4	750	+200
	Mean	40 7	+3.3	8 4	+ 2 5	690	+ 95

ing dialysis were functional and reversible. Although the question is important in principle it is not easy to solve in cases of chronic uraemia where slowing due to morphological change and possible functional slowing of NCV probably take place simultaneously.

For this reason we have studied how an acute change in the metabolite concentration in serum associated with renal failure and/or a single haemodialysis session affects the electrophysiological parameters of the peripheral sensory nerves. In our opinion the result supports Nielsen's theory (17, 18) that the slowing of NCV in uraemic intoxication is at least partly functional and reversible.

PATIENTS AND METHODS

Case reports

Case 1 A 17-year-old schoolgirl who had earlier been healthy. She became ill with a gynaecological infection, which led to prerenal azotaemia. When admitted to hospital her serum creatinine was 9 8 mg/100 ml and she was dehydrated. The fluid and electrolyte disturbances were corrected and the serum creatinine value returned quickly to normal. Dialysis treatment was not necessary.

Case 2 A 60-year-old man who had suffered from pulmonary tuberculosis since 1962. He had had antitubercular medication several times, the last course of treatment ending in Jan 1974. Since then without drugs. The tuberculosis became active again in the following autumn and the patient was admitted to a tuberculosis hospital. At that time renal function was normal on the basis of serum creatinine. Antitubercular treatment was initiated with combination medication which included Rifampicin®. Approximately one hour after the first dose of Rifampicin the patient developed a shivering fit, fever and chest pains, acute oliguric renal failure following. The clinical picture was typical of corresponding case histories published earlier (16). The patient was transferred to the renal unit of the hospital for dialysis treatment. One haemodialysis was performed before which serum creatinine was 14 8 mg/100 ml, potassium 7.8 mEq/l. Diuresis rapidly increased and the patient's renal function began to improve. No further dialysis was needed.

Two weeks after admission his creatinine was 1.5 mg/100 ml and urine volume was normal. The antitubercular treatment was continued avoiding Rifampicin. At the time of check-up in Nov 1975 he was treated with

Acta Med Scand 202 433–434 1977

EDITORIAL

Paresis of Cilia—a New Disease Picture

he mechanisms involved in morphological teratol gy are usually quite obscure and even in such istances when we have found the culprit as in ialidomide damage to the fetus the biochemical as s remains unexplained It has always been clear my mind that we must study what the Germans ill Entwicklungsmechanik (developmental biol gy) Not only as a subject of interest for basic zoo- iysiology but also for pure clinical medicine The erman zoologist Spemann was one of the great oneers and in 1935 he received the Nobel prize in edicine The factors that govern the processes of fferentiation from the fertilized egg to the mature tus may sometimes be surprisingly non specific iere are also however very specific organizers at influence the development of certain cells in a ecial direction Erythropoietin is an excellent ex iple of such a substance and I am sure that the al isolation and synthesis of this substance will be eal break through not only in hematology but in neral medicine

Fetal endocrinology has become a science in its n right The same hormones that are active dur ; extrauterine life may also have a strong influ ce on fetal development One example is congeni myxedema in the newborn athyreotic child re the fetal clinical picture is very similar to the ih A number of congenital malformations on the ital organs have been identified as lack of nor l enzyme function at a certain stage in the syn sis of steroid hormone All the different types of maphroditism have been studied with great suc s by such methods An interesting inherited formation gives an externally normal baby girl o has a rudimentary uterus and will never men iate in spite of the fact that she will develop mal breasts This condition is explained by the that she is really a boy with a male omosomal pattern She has testes that produce osterone but she lacks the normal gene that codes for the formation of a substance that is n mally present on cells that should react testosterone In this instance we do not have a disturbed synthesis of an enzyme Another specific protein that is called the testosterone receptor is lacking This substance binds the steroid hormone to the cell membrane and the hormone molecule is taken into the interior of the cell where it may exert its metabolic activity We have seen such a family where one little girl had classical hemophilia A (factor VIII deficiency) Her' chromosome pat tern was male and her development gave a non menstruating person with normal development of the breasts She has a sister' who is non hemophilic but has the same endocrine disturbance This condition is inherited but is only expressed in the male There is no question about the fact that the study of such specific hormone receptors has become one of the most rapidly expanding fields in medicine Not only pharmacology and biochemistry but also internal medicine and oncology may be quoted as heavily implied in such results This last speciality seems to have great practical use for the study of sex hormone receptors for instance in mammary carcinoma

But let us discuss the subject described in the title of this editorial It has recently been found that paresis or paralysis of cilia may be an inborn error of metabolism and cause widespread disturbances in many organs Kartagener's syndrome has been regarded as one of the most enigmatic among inher ited malformations and still it has been explained in a very simple way during the last year This syndrome is of special interest to the pneumologist because the patients develop severe bronchiectasis in later years They also have a tendency to suffer from sinuitis already as children Many different explanations have been suggested without any reli able foundation One of the most popular has been that there is a defect in the immune response

the respiratory tract One symptom seems to be impossible to integrate into the picture namely situs inversus viscerum

A Swedish zoologist Bjorn Afzelius has recently published a paper in Science (vol 193 1976) that seems to give an excellent overall explanation of all these symptoms He studied four subjects Three of these had a Kartagener syndrome All four produced spermatozoa that were alive but did not move The tail was straight and stiff Electron microscopy showed that the tails did not have the right pattern indicating that they lacked dynein This substance seems to be responsible for the movement not only of the sperm but also of all normal cilia The author studied tracheobronchial clearance in these three subjects and found that there was no measurable transport if the patient did not cough Biopsy material from the bronchial mucosa did not show any ciliary movement and electron microscopy seemed to show that the cilia were abnormal regarding dynein structure Three of the subjects had situs inversus totalis The fourth is the brother of one of the others

It seems to me that this is the final explanation of the syndrome as far as the respiratory symptoms and sterility are concerned It is interesting that sterility does not seem to be mentioned as a symptom in earlier publications A recent paper by Holmes et al contains some pedigrees regarding Kartagener families It is clear from these pictures that several female carriers have had offspring whereas no male patient had children It may well be that this has been regarded as the result of the severe somatic illness Much more probable is the explanation that the paresis of the spermatozoa is the real cause

Afzelius discusses the fact that differentiating cells of vertebrate embryos carry cilia It is possible that the movement of cilia in the early embryo may have a fixed beat direction and that this is instrumental in determining the dextral rotation of the viscera that we see in the normal man Situs inversus is a sinistral instead of a dextral spiral Experimental evidence that this may be true has been obtained on starfish larvae

Family data have shown that there is a group of patients with a partial syndrome who do not have situs inversus This is probably as common as the total possibly indicating that chance alone determines rotation in these persons one half being sinistrally one dextrally rotated

These studies clearly indicate the close connection between clinical medicine and developmental biology It seems possible that ciliary paresis without the complete syndrome is a more common cause of repeated respiratory infections than we have suspected

Jan G Waldenstrom

Thanks to Our Referees

ring the years when I have been Chief Editor this journal the referee system has been used stematically The Editors want to express their atitude towards the many colleagues who have ven time and thought to this task We hope that we shall have the collaboration of these and other specialists within different fields also for future volumes of our journal Through their efforts many faults have been discovered and many obscure points clarified

Aas
J Abdon
Almqvist
Aurell
T Axelsson
Barány
Bauer
Bechgaard
Beermann
Belfrage
Bengtsson
Bengtsson
Bengtsson
Berfenstam
Berg
E Bergentz
Berggård
Berglund
Bergström
Berlin
Bertler
Birke
G Biorck
T Bjurö
V Björk
S E Björkman
P Björntorp
E M Blegen
L O Brandt
O J Broch
T Brundin
K Brøchner Mortensen
L A Carlson
E Cerasi
S Cronberg
O Dahlbäck
A Dahlqvist
G Dahlström
J Dedichen
J Dymling
O Edhag
I Edler
S Effendić
L-G Ekelund
H Eliasch
D Elmqvist
E Enger
L Engstedt
L R Erhardt
S Eriksson
V Faber
B Fagrell
U de Faire
C O Forssman
H Forssman
G Gahrton
L Garby
S Gardell
J Georg
E Gjone
C G Groth
A Gustafsson
H Gøtzsche
K Gydell
K Hall
L Hallberg
P Halonen
B Hamrin
L Å Hansson
M Harboe
R Hed
A H
A J Hellem
C Hellström
G Helmers
E Hess Thaysen
J Hess Thaysen
Å Hjalmarson
S Hofvendahl
G Holm
J Hällén
C Högman
B Hökfelt
O Hook
D Ingvar
K Iversen
J G Jeppsson
B W Johansson
H Jørgensen
L Kaijser
W Kaipainen
G Karpe
A Killander
S Aa Killmann
S Kistner
B A Lamberg
L G Larsson
C B Laurell
T Leonhardt
T Lindholm
S Lindstedt
J E Lindsten
F Linell
J G Ljunggren
R Luft
T Lundberg
B Lundh
S Löfgren
G Magnusson
J Malmquist
H Malmros
G Matell
V Mutt
H Möller
E A Nikkila
B Nilsson
I M Nilsson
B Nosslin
H Odeberg
S B Olsson
E Orinius
J Palmblad
B Pernow
V Posborg Petersen
J Poulsen
H Reichard
P Reizenstein
N Rehnqvist
H Rorsman
A Rosén
B Scherstén
M Schwartz
Å Senning
H Sjöberg
A Sjögren
B Sjogren
F Sjöqvist
U Smith
O Storstein
A Svanborg
N Söderström
B Tengroth

J Thorell
O Thulesius
G Tibblin
E Trell
A Tybjærg Hansen
N Tygstrup
N Törnblom
J Wahren
J Waldenstrom
I Wallentin
H Vallin
O Wegelius
C H de Verdier
L Werko
G Westberg
P O Wester
B Wikland
L W Wilhelmsen
F Wollheim
O Zettervall
H Åberg

It is our hope that the referees will continue to help us to raise the standard of the Acta and we believe that this journal—and thus its readers—have profited greatly from the suggestions and positive criticism

Anonymity has been a condition for unbiased work as a referee A list of their names gives us a chance to remember their efforts without unmasking the individual

Acta Medica Scandinavica thanks our many competent referees and wishes them, as well as authors and readers a Happy New Year 1978

Jan G Waldenstrom

Acta Med Scand 202 437–443, 1977

Dıgıtalıs Therapy ın a 70-Year-Old Populatıon

S Landahl B Lındblad S Roupe B Steen and A Svanborg

From the Department of Genatric and Long Term Care Medicine and the Department of Clinical Chemistry University of Gothenburg Gothenburg Sweden

ABSTRACT In the population study "70-year-old people in Gothenburg" 14% of the probands were found to be undergoing treatment with digitalis, 6% with digoxin, 6% with digitoxin and 2% with other glycosides A comparison between results of the interview method and those of S-digoxin analyses indicates that the interview method was acceptable As far as can be judged from S-digoxin analyses, only about 60% of the treated patients were on a dosage considered to be effective and free from obvious risks of side effects Out of the 130 70-year olds who were on digitalis treatment, 37% had obvious symptoms of heart disease requiring such treatment, 34% lacked symptoms of arrhythmia and/or congestive failure but had heart volumes larger than those used as reference values in younger age groups, and 29% had no symptoms indicating digitalis treatment At least 13% of the population had indications for digitalis therapy and about 75% of those apparently needing digitalis were on such treatment Thus both over and underdiagnosis of heart disease requiring digitalis therapy were common in this age group

The main indications for treatment with digitalis are insufficiency of the myocardial contraction capacity and certain cardiac arrhythmias It is therefore natural that digitalis therapy is common at high age In a county in Sweden about 18% of the population of 70–79 year-olds were reported to be on digitalis therapy (2) Studies in other countries have reported figures varying from about 5% in elderly people at home (6 32) to about 20% in hospitalized patients of all ages (1 18)

Although digitalis therapy has been used for more than a century it is still difficult to handle especially in elderly patients Present knowledge of indications dose requirement dose tolerance side effects and risks of complications in the elderly is rather vague At high age symptoms like dyspnoea and lowered physical capacity may indicate heart disease but may also be signs of normal ageing processes which presumably are not influenced by digitalis therapy A changed susceptibility to digitalis at high age might be due to e g altered intestinal absorption (7 10 21) drug interaction and binding capacity of plasma proteins (14 27) reduced body cell mass (5 25) lowered potassium or magnesium concentrations (12 17 26) altered hepatic (15 16) and renal function (3 19 25 30 31) advanced heart disease (1 4 18) and chronic or acute pulmonary disorders (1 8)

In a broad survey of the social and medical conditions of 70-year-old people in Gothenburg Sweden (20 29) a thorough record was obtained of drug consumption Besides registering this consumption during an interview analyses of drug concentrations in plasma were performed to control the reliability of the interview technique and to evaluate dose response dose tolerance and frequency of under and overdosage The present report is restricted to observations concerning treatment with digitalis in 70-year-old people The data also permit conclusions concerning indications for digitalis therapy in patients at this age as well as the patient s adherence to the doctor's instructions when a dangerous drug like digitalis is prescribed

STUDY POPULATION AND METHODS

The population study 70-year-old people in Gothenburg is a cross sectional study which will be continued prospectively The first sampling was carried out in 1971–72 and the next is due in 1976–77 The sample comprised 1 148 persons of both sexes i e about 30% of the 70-year-old population in Gothenburg The general design study the sampling procedure the general

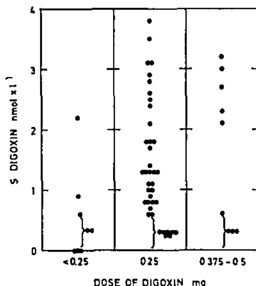


Fig 1 S digoxin concentrations at various daily maintenance doses of digoxin

data collection the analyses of intra and interobserver variation and comparisons between responders (85%) and non responders (15%) have been described previously (20)

The examination consisted of two parts a home call by a registered nurse and a medical examination at the Out Patient Department of Vasa Hospital The home call centered on an interview about social conditions The probands were also told to place every bottle or other package containing drugs on the table and the nurse then interviewed the probands about their actual drug consumption and compared it with the prescribed dosage (13) The clinical data used emanate from the medical examination of the probands which also included ECG recordings were coded according to the Minnesota Code (24) volume was determined roentgenologically according to the method described by Jonsell (11) Methods used for sampling and storage of blood have been reported previously (20) Blood samples were taken not less than 12 hours after the last intake of digitalis

S-digoxin concentrations were determined on serum from 58 of the 59 patients who claimed that they were being treated with digoxin and from the first 58 probands claiming that they did not use digitalis The latter were used as controls No proband had had a prescribed change in the dosage of digoxin within the last month The determinations were carried out in duplicate with a solid phase radioimmunoassay kit from Clinical Assay Inc USA (Gammacoat Lot D 003) The precision in the analyses as estimated by the coefficient of variation in duplicate was with this lot 15% (mean 1 8 nmol/l $n=74$) No values below 0 6 nmol/l were used in this calculation of precision Results from S-digoxin analyses significantly different from the zero standard but with values below 0 6 nmol/l were not evaluated numerically but were taken together in the group 0 1–0 5 nmol/l

All the 130 patients on digitalis who took part in the medical examination were compared with the other probands concerning indications for digitalis treatment symptoms and signs that might be consistent with over dosage of digitalis and the existence of risk factors for digitalis intoxication

The following criteria were used as indications for digitalis treatment 1) Roentgenological evidence of increased heart volume plus two of the symptoms oedema, dyspnoea and cyanosis 2) All the three symptoms oedema dyspnoea and cyanosis 3) Roentgenological evidence of pulmonary congestion 4) Atrial flutter or fibrillation 5) Roentgenological evidence of increased heart volume in the absence of systemic hypertension

In patients treated with digitalis which might have decreased the heart volume a heart volume of at least 450 ml/m² BSA in males and at least 400 ml/m² in females was taken as evidence of cardiac enlargement while at least 500 and 450 ml/m² respectively was used in those without digitalis treatment Systemic hypertension was defined as a casual diastolic BP of at least 115 mmHg (phase 4) or an anamnestic statement of treatment for hypertension

As symptoms which might indicate overdosage of digitalis were recognized fatigue reduced appetite naus and vomiting loose faeces AV block in ECG ventricu premature beats and bradycardia

One or more of the following symptoms or signs we considered to increase the risk of side effects or intoxication Ischemic heart disease as defined by Rose (2 2 Chronic bronchitis as defined by WHO (morning cou during at least 3 months a year) Pulmonary emphyse as evidenced by observations at chest X ray S prote S potassium or S magnesium values below the avera of the total group of 70-year-olds S bilirubin S AL S-ASAT S-ALAT S creatinine or S urea above the ave age A lower body weight than the average of the 7 year olds

RESULTS

Almost 14% of the 1 007 probands taking part in t home call stated that they used digitalis At t interview, 5% of the digitalis treated proban stated that they used less digitalis than prescribe Six per cent used digoxin and 6% digitoxin prepar tions (Table I) In this group of digitalis treat

Table I *Males and females (%) using cardi glycosides*

	Males ($n=470$)	Females ($n=537$)
Digoxin	6 6	5 6
Digitoxin	5 9	5 6
Acetyldigitoxin	0 2	0 4
Lanatocid C	0 2	1 1
Proscillaridin A	0 2	0 6
Adonisglycosides	0 2	0 6
Total	13 4	13 8

Table II *Probands (%) presenting indications for digitalis treatment*

1=Heart enlargement plus 2 of the symptoms dyspnoea cyanosis or oedema 2=all the three symptoms dyspnoea cyanosis and oedema 3=roentgenological evidence of pulmonary congestion 4=atrial flutter or fibrillation 5=heart enlargement in the absence of hypertension

	Criterion						
	1	2	3	4	5	Any of 1–4	Any of 1–5
Untreated (n=841)	3	0 5	1	1	16	6	18
Digoxin (n=59)	24	5	9	10	61	34	68
Digitoxin (n=60)	23	15	7	18	63	43	77
Other glycosides (n 11)	9	0	0	9	46	18	55
All digitalis treated (n=130)	22	9	7	14	61	37	71
All probands (n=971)	6	2	2	3	22	10	25

patients 49% were also being treated with diuretics

The average daily digoxin dose was 0 26±0 07 mg (mean±S D) and the daily maintenance dose for digitoxin treated patients was 0 1±0 028 mg (mean ±S D) Of the 58 digoxin treated patients 1% had no measurable serum concentration Serum concentrations of 0 1–0 5 nmol/l were found in 21% and above 2 9 nmol/l in 10% The highest value recorded was 3 8 nmol/l

The digoxin concentration in serum varied markedly even among patients on the same dosage (Fig 1) Thus the levels in patients receiving 0 25 mg digoxin per day varied from below 0 6 to 3 8 nmol/l The group of 5 patients with no measurable digoxin concentration in serum included the two digoxin treated patients who stated that they used less digitalis than prescribed and only when they found it necessary Two probands with no digoxin in serum claimed that they used 0 125 mg per day and one 0 25 mg six days per week

Concerning the indications for digitalis therapy 18% of the probands without digitalis and 71% of the digitalis treated patients met with at least one of our five criteria (Table II) The majority of the probands meeting with our criteria for digitalis treatment had no other symptoms of congestive heart failure than an increased heart volume If heart enlargement as the sole symptom or sign is excluded as an indication for digitalis treatment only 6% of the untreated and 37% of the digitalis treated patients i e 10% of all probands had indications for digitalis treatment There was no significant difference in the occurrence of indications for digitalis treatment between patients with values in the upper (>2 1 nmol/l) and the lower (<0 6 nmol/l) quartile of S-digoxin concentrations

Table III shows the frequency of symptoms and signs that might indicate overdosage of digitalis In the digoxin treated group significantly more patients had one of the symptoms fatigue AV block I ventricular premature beats ($p<0\ 001$ χ^2 test) and a reduced appetite ($p<0\ 01$ χ^2 test) than probands without digitalis There was no significantly positive correlation between the occurrence of these symptoms and the S digoxin concentrations since patients with S-digoxin above 2 1 nmol/l did not have at least two of the symptoms more often than those with S-digoxin below 0 6 nmol/l However there was a tendency for a higher frequency of anorexia and fatigue to accompany a higher S-digoxin concentration

Table III *Probands (%) with symptoms consistent with digitalis overdosage*

	Untreated (n=841)	Digoxin-treated (n=59)	Digitoxin treated (n=60)
Fatigue	11	32***	23**
Reduced appetite	3 6	12 *	13***
Nausea	2 1	3 4	10
Loose faeces	7 0	12	6 7
AV block II+III	0 1	0	1 7
AV block I	3 0	12	10
Premature ventricular beats	1 2	6 8 *	1 7
Heart frequency <50	0 7	1 7	3 3
2 or more symptoms	7 6	24 *	25**

**$p<0\ 01$ **$p<0\ 001$ (χ^2 test against untreated)

Table IV *Frequency of symptoms suggestive of digitalis overdosage in 149 probands without digitalis treatment but with indications for such treatment*

	% of total no of subj
Fatigue	10
Reduced appetite	4 7
Nausea	3 4
Loose faeces	4 7
AV block II+III	0 7
AV block I	8 1
Premature ventricular beats	2 0
Heart frequency <50	*1 3*
2 or more symptoms	5 4

Significantly more digitoxin treated patients reported loss of appetite nausea ($p<0\ 001$, χ^2 test) fatigue or had AV block I ($p<0\ 01$, χ^2 test) than those not treated with digitalis No difference in the frequency of these symptoms was found between patients on digoxin or digitoxin treatment A comparison between probands meeting with our criteria for digitalis treatment but without digitalis therapy and those on digitalis showed that significantly more of the digitalis treated probands reported fatigue (10 and 27% respectively $p<0\ 001$ χ^2 test) reduced appetite (5 and 12% respectively $p<0\ 05$ χ^2 test) and two or more of the symptoms regarded as signs of intoxication (5 and 25% respectively, $p<0\ 001$ χ^2 test) (Table IV)

Significantly more digitalis treated patients than without digitalis had the following conditions probable ECG evidence of ischaemia as defined by Rose (23) (18 and 4% respectively, $p<0\ 001$ χ^2 test) and angina pectoris as defined by Rose (22) (8 and 4% respectively $p<0\ 05$ χ^2 test) (Table V) There was no difference in the frequency of these symptoms between patients treated with digoxin or digitoxin We did not find coronary heart disease or chronic pulmonary disease more often among the digitalis treated patients with two or more symptoms of overdosage than in patients without such symptoms

The S creatinine level in the total population of 70-year-olds was 79±20 μmol/l (mean±S D) Patients with S digoxin concentrations above 2 1 nmol/l had significantly higher creatinine values than patients with S digoxin levels below 0 6 nmol/l (97±25 and 79±12 μmol/l, respectively $p<0\ 05$ Student s t test) The patients with S-digoxin above 2 1 nmol/l had a lower average body weight (66±16 6 and 75±15 6 kg respectively) and a higher S-urea value (6 6±1 5 and 5 6±1 3 mmol/l respectively) but these differences were not significant (Table VI)

We found only two significant differences in the laboratory results between digitalis treated patients with two or more symptoms which might be due to overdosage and those without these symptoms i e the digoxin treated patients with symptoms of overdosage probably had a higher (sic!) S potassium (4 0±0 4 and 3 7±0 3 mmol/l respectively, $p<0\ 05$ Student s t test) and a more pronounced enlargement of the heart (519±134 and 438±89 ml/m² BSA respectively $p<0\ 05$ Student s t test) (Table VII) Neither in the digoxin nor in the digitoxin treated probands did we find any differences in the frequency of symptoms and signs of overdosage between the lower and upper quartiles of laboratory data listed in Table VII

DISCUSSION

The fact that none of the controls had any measurable amounts of digoxin in serum while measurable amounts were found in 91% of patients reporting digitalis treatment indicates that the interview method used to investigate digitalis consumption among the 70 year olds was acceptable

Of the probands on prescribed digoxin 29% had serum levels below 0 6 nmol/l and 10% above 2 9 nmol/l As far as can be judged from the digoxin analyses only about 60% of the treated patients were on a dosage considered to be effective and without obvious risks of side effects

Clinical experience indicates that it is more dif

Table V *Percentage of probands with coronary heart disease and/or chronic pulmonary disease*

	Un treated (n=841)	Digoxin treated (n=59)	Digitoxin-treated (n=60)
LBBB	1 5	1 7	1 7
RBBB	2 6	3 4	8 3*
Probable ischaemia	4 4	20***	17**
Angina pectoris	4 0	8 5	8 3
Chronic bronchitis	13	8 5	15
Pulmonary emphysema	3 1	1 7	1 7

*$p<0\ 05$ ***$p<0\ 001$ (χ^2 test against untreated)

Table VI *Results from some laboratory tests and body weight (mean±S D) grouped according to S-digoxin concentration*

	S-digoxin (nmol/l)			
	<0 6 (n=16)	0 6–1 0 (n=14)	1 1–2 1 (n=14)	2 2–3 8 (n=14)
S-creatinine (μmol/l)	79±12	79±17	97±32	97±25
S-urea (mmol/l)	5 6±1 31	5 8±1 64	6 9±2 54	6 6±1 51
S-K (mmol/l)	3 8±0 24	3 8±0 46	3 9±0 51	3 6±0 44
S-Ca (mmol/l)	2 4±0 16	2 4±0 15	2 4±0 06	2 5±0 10
S-Mg (mmol/l)	0 9±0 11	0 9±0 10	0 9±0 09	0 9±0 08
B wt (kg)	75±15 6	73±12 8	65±12 8	66±16 6

ficult to predict the digoxin requirement in elderly than in young people There was a considerable variation in digoxin levels among patients receiving the same daily maintenance dose Similar variations also occur in young patients (4 21) and our figures do not allow the conclusion that such variations are more pronounced in the elderly The elderly probably need a lower dosage of digoxin than young people (5 18) and might thus be more susceptible to digitalis As we do not know of similar studies to the present one performed on younger populations it has not been possible to compare the doses used by these 70 year olds with those used by younger population samples Our own experience as well as that reported by many others (5 9 18) strongly indicates that the dose tolerance for digitalis is in fact lower in the elderly One reason is apparently the common changes in renal function at high age These changes are often not reflected in alterations in S creatinine levels making them difficult to estimate in clinical routine Renal impairment especially influences the dose tolerance of digoxin (3 9 25 30 31) In the present study however no difference in the frequency of symptoms which might indicate digitalis overdosage was observed between patients treated with digoxin and digitoxin respectively Another reason for a lowered dose tolerance in the elderly might be age related changes in body cell mass body water etc Prospective studies of representative groups at high age have not yet been performed As far as can be judged from cross sectional population data (28) males have a lower body cell mass at 70 than at 54 years of age but significant changes in body composition probably do not occur in females until after the age of 70 Age related changes in the susceptibility to digitalis

Table VII *Laboratory results and heart rate among digitalis treated patients with and without symptoms indicating overdosage (mean±S D)*

	Digoxin treated		Digitoxin treated	
	≥2 symptoms (n=16)	No symptoms (n=18)	≥2 symptoms (n=12)	No symptoms (n=25)
S bilirubin (μmol/l)	11±3 1	9 2±2 9	8 7±2 6	9 9±2 2
S-ALP (μkat/l)	1 4±0 48	1 4±0 41	1 5±0 77	1 5±1 6
S ASAT (μkat/l)	0 39±0 08	0 56±0 55	0 47±0 48	0 50±0 27
S ALAT (μkat/l)	0 25±0 09	0 31±0 14	0 44±0 22	0 42±0 26
S-K (mmol/l)	4 0±0 40	3 7±0 30	3 7±0 40	3 6±0 30
S-Ca (mmol/l)	2 4±0 10	2 4±0 10	2 4±0 15	2 4±0 15
S-protein (g/l)	79±5 9	77±3 6	80±7 4	81±8 7
S-creatinine (μmol/l)	88±26	79±18	79±18	79±18
S-urea (mmol/l)	7 4±3 0	5 9±2 1	6 3±2 6	6 0±2 1
Heart volume (ml/m²)	519±134*	438±89	425±77	481±97
Heart rate	76±15	84±8	78±11	81±7

* $p<0\ 05$ Student s t test

are thus caused not only by diseases common at high age but also by normal ageing processes (4 5 9, 21) It is not known to what extent increased susceptibility to digitalis occurs successively during the later part of life or begins at a high age when changes in e g , body composition physical capacity and prevalence of disease become more rapid

The altered pharmacokinetics of digitalis in the elderly causes a high risk of digitalis intoxication Symptoms of digitalis intoxication have been reported to occur in 5–35% of hospitalized patients on digitalis therapy and the mortality directly attributable to toxic cardiac effects of digitalis seems to be unacceptably high in many patient groups (1 4 9) There was a significant correlation in our study between digitalis treatment and certain symptoms and signs often associated with digitalis intoxication viz fatigue nausea a reduced appetite AV block I and ventricular premature beats occurring about three times as often in patients treated with digitalis than in those without such treatment Some of these symptoms might be due to the heart disease per se A comparison between probands without digitalis but meeting with our criteria for digitalis treatment and those treated with digitalis showed a significantly higher frequency of fatigue reduced appetite and two or more symptoms of overdosage among the digitalis treated This might indicate that the figures obtained really do indicate the frequency of overdosage of digitalis i e that such overdosage in about 15–20% of the patients treated i digitalis There was no difference between di in and digitoxin as far as symptoms of over were concerned It has been suggested earlier that digitoxin treatment should cause a lower incidence of intoxication than digoxin (9) probably mainly because the pharmacokinetics of digitoxin is less dependent on renal function We however hesitate to draw conclusions concerning the absolute frequency of digitalis overdosage in this material as no significant difference in the concentration of digoxin in serum was observed between patients with symptoms of overdosage and those without Pronounced hepatic and renal insufficiency and electrolyte disturbances that might be expected to influence digitalis tolerance were rare in this population

The present observations strongly emphasize the need for a more thorough individualization of digitalis dosage Obviously a registration of general effects of digitalis is more important for adequate dose adjustment than S-digoxin analyses which seem to be of value mainly for assessing symptoms and signs that might be due to both over and underdosage of digitalis

Signs associated with normal ageing e g effort dyspnoea and a lowered physical capacity are apparently sometimes used as indications for digitalis treatment Approximately 30% of those treated with digitalis did not have any symptoms or signs of heart disease indicating digitalis therapy and about 35% of the digitalis treated patients did not have other symptoms of heart insufficiency than heart enlargement We have found it meaningful to draw attention to the figures that include probands with enlargement of the heart as the only symptom of cardiac failure as treatment with digitalis and diuretics often eliminates symptoms like dyspnoea and oedema

The possibility that the ageing process per se might cause a slight increase in heart volume must be taken into consideration but data on normal heart volume at high age are lacking at present Further processing of our data from the present population study will allow conclusions concerning the heart volume in probands without obvious signs of heart disease and/or systemic hypertension Furthermore certain symptoms e g dyspnoea oedema and cyanosis may have had other causes than heart disease

At the present stage in the processing of this material from the population study of 70 year-olds in Gothenburg our observations indicate that at most 25% and at least 13% of the population needed digitalis therapy at this age and that about 14% of the population received such therapy At least 30% of the digitalis treated probands had no actual symptoms indicating digitalis therapy It is obvious that in many of these probands digitalis therapy probably had been initiated without proper indications In some of the probands a definite evaluation of possible indications for digitalis therapy could not be made as long as digitalis treatment was being given Furthermore only about 2/3 of those apparently needing digitalis therapy were on such treatment as 6% of probands without digitalis therapy had definite symptoms of cardiac failure or arrhythmias that should be treated with digitalis

Thus our study shows that both overdiagnosis and underdiagnosis of heart disease requiring digitalis therapy were common in this age group

REFERENCES

1 Beller G A Smith T W Abelmann W H Haber E & Hood W B Digitalis intoxication N Engl J Med 284 989 1971

2 Bergstrom J Boethius G & Wiman F Sjuk domspanorama utlast ur en läkemedelsregistrering Lakartidningen (Suppl) IV 34 1972

3 Doherty J E The influence of renal function on digoxin metabolism In Symposium on digitalis (ed O Storstein) p 168 Gyldendal Norsk Forlag Oslo 1973

4 Evered D C & Chapman C Plasma digoxin con centrations and digoxin toxicity in hospitalized pa tients Br Heart J 33 540 1971

5 Ewy G A Kapadia G G Yao L Lullin M & Marcus F J Digoxin metabolism in the elderly Circulation 39 449 1969

6 Gibson J & O Hare M Prescription of drugs for old people at home Gerontol Clin 10 271 1968

7 Goldfinger S E Heizer W D & Smith T W Absorption of digoxin in patients with malabsorption In Symposium on digitalis (ed O Storstein) p 224 Gyldendal Norsk Forlag Oslo 1973

8 Hargreave F E Digitalis and cor pulmonale Br Med J 2 943 1965

9 Hillestad L Hansteen V Hatle L Storstein L & Storstein O Digitalis intoxication In Symposium on digitalis (ed O Storstein) p 281 Gyldendal Norsk Forlag Oslo 1973

10 Huffman D H & Azarnoff D L Absorption of orally given digoxin preparations JAMA 222 957 1972

11 Jonsell S A method for the determination of the heart size by teleroentgenography Acta Radiol 20 325 1939

12 Jørgensen A W & Sørensen O H Digitalis intoxi cation Acta Med Scand 188 179 1970

13 Landahl S & Steen B Lakemedelskonsumtion hos 70-åringar i Goteborg Lakartidningen 72 5158 1975

14 Lukas D S Binding of digitoxin digoxin their ge nins and related digitoxosides in human plasma pro tein In Symposium on digitalis (ed O Storstein) p 31 Gyldendal Norsk Forlag Oslo 1973

15 — The role of the liver in chemical transformation of digitoxin In Symposium on digitalis (ed O Stor stein) p 192 Gyldendal Norsk Forlag Oslo 1973

16 Marcus F J The role of the liver in the transforma tion and excretion of digoxin Symposium on digitalis (ed O Storstein) p 200 Gyldendal Norsk Forlag Oslo 1973

17 Neff M S Mendelssohn S Kim K E Banach S Schwartz C & Seller R H Magnesium sulfate in digitalis toxicity Am J Cardiol 29 377 1972

18 Ogilvie R J & Ruedy J An educational program in digitalis therapy JAMA 222 50 1972

19 Rasmussen K Jervell J Storstein L Gjerdrum K Digitoxin kinetics in patients with impaired renal function Clin Pharmacol Ther 15 6 1972

20 Rinder L Roupe S Steen B & Svanborg A 70-year-old people in Gothenburg A population study in an industrialized Swedish city I General design of the study Acta Med Scand 198 397 1975

21 Roe P F & Abbot A L Studies in serum digoxin Age Ageing 3 106 1974

22 Rose G A The diagnosis of ischemic heart pain and intermittent claudication in field surveys Bull WHO 27 645 1962

23 — Chest pain questionnaire Milbank Mem Fund Q 43 32 1965

24 The Scandinavian Committee on ECG classification The Minnesota Code or ECG classification adapta tion to CR leads and modification of the code for ECG s recorded during and after exercise Acta Med Scand (Suppl) 481 1967

25 Schapel G J McGrath B P Edwards K D G Hawkins M R & Mitchell A S Therapeutic serum digoxin concentration Relation to age weight sex and serum creatinine level Aust NZ J Med 3 606 1973

26 Seller R H The role of magnesium in digitalis toxic ity Am Heart J 82 551 1971

27 Solomon H M & Abrams W B Interactions be tween digitoxin and other drugs in man Am Heart J 83 277 1972

28 Steen B Bengtsson C Bruce Å Isaksson B & Tibblin G Kroppssammansattningen hos man och kvinnor i olika åldrar i Goteborg Resultat från tre populationsundersökningar In Nordisk gerontologi Forhandlingar från Andra Nordiska Kongressen i Gerontologi 4–7 maj 1975 (ed B Steen & A Svanborg) p 213 Astra Sodertalje 1976

29 Steen B & Svanborg A Halsoundersokningen 70-åringar i Goteborg Upplaggningen i stort Lakartidningen 70 957 1973

30 Steiness E Renal excretion of digoxin In Symposium on digitalis (ed O Storstein) p 178 Gyldendal Norsk Forlag Oslo 1973

31 Storstein L The influence of renal function on the pharmacokinetics of digitoxin In Symposium on dig italis (ed O Storstein) p 158 Gyldendal Norsk Forlag Oslo 1973

32 Taylor B B Kennedy R D & Caird F J Digoxin studies in the elderly Age Ageing 3 79 1974



Acta Med Scand 445–447 1977

Studies on Digitalis

XIV Is there any Correlation between Hypomagnesemia and Digitalis Intoxication?

Ole Storstein Viggo Hansteen Liv Hatle Leif Hillestad and Liv Storstein

From University Hospitals Rikshospitalet Medical Department B Ullevål Hospital Medical Department VIII Oslo and Akershus Central Hospital Medical Department Nordbyhagen Norway

ABSTRACT In a prospective study on digitalis intoxication low serum magnesium was found in 90 patients, while 388 patients had values above 1 5 mEq/l Hypomagnesemia was more frequent in women than in men, in those with low body weight and in those with advanced heart failure More patients with hypomagnesemia than those without had nausea, anorexia, fatigue, flickering of vision and atrial tachycardia with block Patients with hypomagnesemia also had lower serum potassium than normomagnesemic patients There was, however, no significant difference in the prevalence of digitalis intoxication or in serum digitoxin concentration Nor was there any correlation between serum digitoxin and serum magnesium levels

Magnesium is present in the cells in amounts second only to potassium It is therefore conceivable that magnesium might be of some importance for the action potential of the myocardial cell Hypomagnesemia has been found to shorten the effective refractory period and to increase ventricular vulnerability (11) Furthermore digitalis intoxication both in experimental and in clinical studies has been reported to increase the incidence of arrhythmias in hypomagnesemic patients (4 6 8) The mechanism is supposed to be that digitalis inhibits cell membrane ATPase activity resulting in decreased intracellular potassium As magnesium is a coenzyme that activates ATPase it is likely that magnesium deficiency contributes to a greater intracellular potassium loss which sensitizes the myocardium to digitalis

In a prospective study on digitalis intoxication (9) we found that hypomagnesemia was more prevalent in intoxicated than in non intoxicated patients and further that hypomagnesemia was the only electrolyte disturbance found in digitalis intoxicated patients Hypomagnesemia was found in 30% of toxic patients as against 18% in non toxicated patients

We therefore wanted to compare patients with low and normal serum magnesium to find out if they differed with regard to clinical and laboratory findings which might indicate digitalis intoxication and if the hypomagnesemic patients showed a higher prevalence of digitalis intoxication

PATIENTS AND METHODS

Of the 649 patients in the study on digitalis intoxication serum magnesium was determined in 478 using the method of atomic absorption spectroscopy A comparison was made between the characteristics of patients with serum magnesium levels below 1 5 mEq/l ($n=90$) and those with serum magnesium above 1 5 mEq/l ($n=388$) Paired observations were analyzed statistically by Student's t test

RESULTS

A great many parameters which might indicate digitalis intoxication were recorded Table I gives only those which showed any statistically significant difference A complete list of the parameters recorded is given elsewhere (9)

Hypomagnesemia was significantly more common in women than in men ($p<0.03$) The patients with low serum magnesium also had

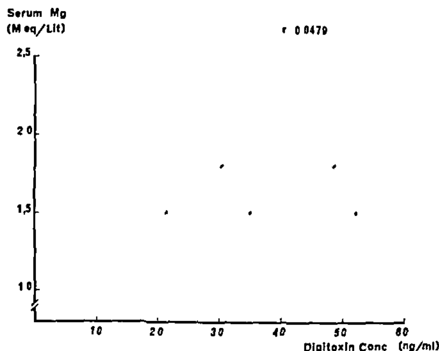


Fig 1 Correlation between serum magnesium and digitoxin concentration in patients with digitalis intoxication

weight than the normomagnesemic ($p<0.02$) Furthermore patients with advanced heart failure (functional classes III and IV) had a higher prevalence of hypomagnesemia than patients i functional classes I and II ($p<0.05$)

Nausea anorexia extreme fatigue and flickering of vision were more common in hypomagnesemic than normomagnesemic patients These symptoms are, however non specific Nausea and anorexia may be the cause of hypomagnesemia and not necessarily a symptom of low serum magnesium

The incidence of atrial tachycardia with block was somewhat higher in hypomagnesemic than normomagnesemic patients ($p<0.03$) Otherwise there was no difference in the incidence of various ECG changes suggesting digitalis intoxication

ble I *Some characteristics of patients with low and normal serum magnesium*

Characteristics	Serum magnesium levels (mEq/l) <1 5 (n=90)	>1 5 (n=388)	p value
Male/female ratio	44 4/55 6	57 8/42 2	<0 03
Weight (kg)	63 4±13 0	66 9±11 4	<0 02
NYHA functional class			
I & II	43 2	59 8	
III & IV	56 9	40 2	<0 005
Nausea (%)	10 2	3 4	<0 02
Anorexia (%)	17 8	5 4	<0 001
Extreme fatigue (%)	6 7	2 1	<0 04
Flickering of vision (%)	6 7	2 3	<0 05
AT with block (%)	5 6	1 3	<0 03
Serum potassium	4 2±0 5	4 4±0 5	<0 04
Serum magnesium	1 3±0 1	1 7±0 2	<0 0001
Digitalis intoxication	9 (10%)	22 (6%)	
No digitalis intoxication	81	366	NS
Serum concentration of digitoxin (ng/ml)	17 5±9 0	16 4±7 8	NS

NS=not significant

Apart from serum magnesium only serum potassium showed lower values in hypomagnesemic than in normomagnesemic patients ($p<0.04$)

Digitalis intoxication was seen more frequently in hypomagnesemic (10%) than normomagnesemic patients (6%) but the difference was not statistically significant Nor was there any significant difference in serum digitoxin concentration between the two groups Fig 1 shows that there was no correlation between serum magnesium and digitoxin concentrations ($r=0.0479$)

DISCUSSION

Apart from the use of diuretics, anorexia and a low intake of food may explain the low serum magnesium found in the present patients The findings of a higher prevalence of hypomagnesemia among women a lower body weight in those with low serum magnesium and more severe cardiac failure (functional classes III and IV) in patients with hypomagnesemia corroborate the findings from the study on digitalis intoxication as these characteristics were also more common in digitalis intoxicated patients

Kim et al (4) found a low serum magnesium concentration 1.38 ± 0.30 mEq/l in 21 patients with digitalis intoxication After correction of the intoxication serum magnesium rose to 1.58 ± 0.16 mEq/l Beller et al (1) found hypomagnesemia in 21% of their patients with and in 10% of those without digitalis toxicity They did not however find any correlation between serum magnesium concentration and serum digoxin or digitoxin concentrations Nor did Holt and Goulding (3) find any correlation between magnesium concentration and the incidence of digitalis toxicity In a recent study of digoxin intoxication in children Singh et al (8) found hypomagnesemia in 5 of 9 toxic patients as against 3 of 10 non intoxicated Serum digoxin level in toxic patients was significantly higher than in non toxic ($p<0.05$) In 3 cases magnesium sulfate was successfully used for the management of cardiac arrhythmias It has previously been reported that magnesium may be used in the treatment of digitalis induced arrhythmias (2 5 7 10)

Our study does not show any increased prevalence of digitalis intoxication in hypomagnesemic patients Nor was there any relationship between hypomagnesemia and digitoxin concentration in serum On the other hand hypomagnesemia was seen more commonly in patients exhibiting symptoms and signs of digitalis intoxication This study should at the very least alert the clinician to the occurrence of hypomagnesemia in patients with congestive heart failure especially in those with signs of digitalis intoxication Magnesium supplements may be needed in hypomagnesemic patients especially in those with symptoms and signs of digitalis intoxication

REFERENCES

1 Beller G A Hood W B Jr Smith T W Abelmann W H & Wacker W E C Correlation of serum magnesium levels and cardiac digitalis intoxication Am J Cardiol 33 225 1974
2 Ghani M F & Smith J R The effectiveness of magnesium chloride in the treatment of ventricular tachyarrhythmias due to digitalis intoxication Am Heart J 88 621 1974
3 Holt D W & Goulding R Magnesium depletion and digoxin toxicity Br Med J 1 627 1975
4 Kim Y W Andrews C E & Ruth W E Serum magnesium and cardiac arrhythmias with special reference to digitalis intoxication Am J Med Sci 242 127 1961
5 Lim P & Jacob E Magnesium deficiency in patients on long term diuretic therapy for heart failure Br Med J 3 620 1972
6 Neff M S Mendelssohn S Kim K E Banach S Swartz C & Seller R H Magnesium sulfate in digitalis toxicity Am J Cardiol 29 377 1972
7 Seller R H Cangiano J, Kim K E Mendelssohn S Brest A N & Swartz C Digitalis toxicity and hypomagnesemia Am Heart J 79 57 1970
8 Singh R B Dube K P & Srivastav P K Hypomagnesemia in relation to digoxin intoxication in children Am Heart J 92 144 1976
9 Storstein O Hansteen V Hatle L Hillestad L & Storstein L Studies on digitalis XIII A prospective study of 649 patients on maintenance treatment with digitoxin Am Heart J 93 434 1977
10 Szekely P & Wynne N A The effect of magnesium on cardiac arrhythmias caused by digitalis Clin Sci 10 241 1951
11 Watanabe Y & Dreifus L S Electrophysiological effects of magnesium and its interactions with potassium Cardiovasc Res 6 79 1972

…abolic data (1st and 2nd day)

…atinine (…mol/l)	Urea (mmol/l)	Potassium (mmol/l)
…4	48 4	7 8
…7	34 5	3 8
…4	24 9	5 6
…4	4 3	2 7
…2	25 9	5 1
…	6 6	2 9
…0	31 4	4 4
…6	16 5	4 0

…soniazid and PAS Diuresis and urine sediment were normal and no proteinuria was present Renal function was slightly decreased creatinine clearance 0 95 ml/sec/1 73 m²

Cases 3–5 Three patients undergoing maintenance dialysis treatment one man and two women respective ages 36 44 and 37 years Patient 3 was diagnosed as having chronic interstitial nephritis and dialysis treatment had continued for two months Case 4 had chronic glomerulonephritis and dialysis treatment had continued for 11 months Case 5 had renal hypoplasia and dialysis treatment had continued for 4 months It had not been possible in any of these cases to establish clinically certain polyneuropathy symptoms All dialyses were carried out with a Travenol RSP artificial kidney Each dialysis session lasted 6 hours and an Ex 23 disposable dialyser was used (cuprophan membrane surface area 0 8 m²)

Laboratory methods

The neural action potential (NAP) of the four peripheral sensory nerves of the patients lower limbs was recorded with a bipolar surface electrode on the medial side (N peronaeus) of the M extensor hallucis longus and behind (N suralis) the malleolus lateralis The supramaximal transcutaneous stimulation was carried out 10–15 cm more proximally with the aid of the same type of electrode The NAPs generally 10–20 μV with commercial EMG equipment and summated by an analogue/digital multichannel analyser the minimal time resolution of which had been set at 50 μsec The latency to the first (positive latency I) and second (negative latency II) peak of NAPs was measured with the same degree of accuracy and the peak to-peak amplitude of the NAP was determined with a degree of accuracy of 0 1 μV The length of the nerve segment under measurement (stimulating cathode proximal recording electrode) was measured with the ankle in hyperextension (N peronaeus) or flexion (N suralis) with a degree of accuracy of 1 mm Finally the temperature of the skin was measured with a degree of accuracy of 0 5 C at several points along the segment studied and the average of the temperature measurements was calculated An electric thermometer was used for these measurements

The results were used to calculate the maximal NCV (length of nerve segment per latency I) of the sensory nerve A temperature correction was further made to the value measured on the basis of its NCV temperature regression which was calculated with the normal material of the laboratory By using a method to be explained elsewhere (15) the error in the measurement of NCV that is due to temperature differences is considerably reduced The temperature corrected NCV value is given either 1) as an absolute value 2) as the difference between two successive measurements (Table I) or 3) as the difference from the normal average (Fig 1)

RESULTS

Table I gives a summary of the results before and after dialysis The first measurement was made immediately before commencing dialysis the second on the following day In patient 1 in whom dialysis treatment was not necessary the measurements were carried out on the first and fourth days of treatment Serum creatinine urea and potassium contents were measured from blood samples taken on the same day as the neurophysiological measurements On an average NCVs amplitude of NAPs and their rise times increased in successive measurements For the first two parameters the change is significant ($p<0.005$) and for the rise time indicative ($p<0.05$) (Wilcoxon s matched pair test) There was no significant difference in skin temperature between the different studies and no subcutaneous oedemas were observed in these patients at any stage

It was possible to follow patient 2 for a longer period Fig 1 shows the gradual normalization of the patient s serum creatinine content and daily urine volume during the follow up period after the only haemodialysis and the average increase in amplitude of NAP and the NCV of N suralis the same period The corresponding va

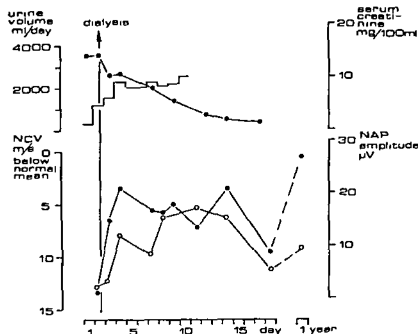


Fig 1 Case 2 Changes in urine volume serum creatinine level average conduction velocity (●) and amplitude of the compound nerve action potential (O) in N suralis during the follow-up period Time days after hospitalization Note conduction velocity given as deviation in m/sec from temperature corrected normal average

peronaeus and rise time for the NAP of all nerves also increased uniformly in successive measurements Improvement of nerve function continued up to the tenth day of observation after which two successive examinations showed a somewhat uniform and symmetrical deterioration of nerve function At this stage the patient was still rather uraemic One year later however his nerve function had already returned to normal

DISCUSSION

In literature about the pathophysiology of uraemic polyneuropathy there are surprisingly few references to the effect of acute renal failure and a single haemodialysis session on the functioning of the peripheral nerves Dayan et al (5) mention that the motor NCV of the Nn poplitei in one of their patients was 40 and 30 m/sec right and left respectively during acute renal failure and four weeks later it was normal This is the only reference to the subject we have found in literature For the effect on NCV of a single haemodialysis session we have found only one systematic series of measurements in a study by Jebsen et al (12) According to these authors a single dialysis session had no major effects on motor nerve or sensory NCVs in a group of 18 patients Their method of investigation differed however in many respects from that used by us Measurements were either motor or concerned only the sensory NCV values of the upper limbs the interval between the first and second measurements was shorter because both measurements were carried out while dialysis was in progress there was no temperature control and computer technique which adds so much to accuracy was not used in the sensory measurements As a matter of fact in the series referred to the sensory NCV of N medianus speeded up significantly ($p<0.01$) during haemodialysis The result would perhaps have been clearer if, for example, the interval between measurements had been long enough for the change in serum metabolite concentration to affect sufficiently the conduction mechanism of the nerves

The slowing down of the NCV that usually occurs in polyneuropathies the decrease in amplitude of the NAP usually connected with it and the distortion of their configuration are explained in two different ways (9) On the one hand the neuropathic process may damage selectively a certain fraction of the peripheral nerve fibres usually the thick myelinated ones with a higher conduction rate When these cease functioning due to axonal degeneration the result is a slight statistical slowing of NCV The other possibility is that the pathological process damages primarily Schwann s cells leading to local often initially paranodal demyelinization which later spreads to the whole internodal segment This delays the depolarization of the membrane potential at the adjacent nodes hence sl

ing the saltatory propagation of nerve impulses Extensive demyelinization may eventually result in a conduction block

In uraemia the block may be limited selectively to the thick rapidly conducting nerve fibres (6) In many polyneuropathies these two histopathological mechanisms occur simultaneously and it is difficult covered in three patients that in the excited fibres uraemic neuropathies (24) Axonal degeneration (1) is probably primary in advanced uraemic neuropathy but after acute renal failure segmental demyelinization dominates the picture (5 7)

Axonal degeneration cannot of course explain a slowing down of NCV which passes within a few days On the other hand it is known that segmental demyelinization develops very rapidly when there is acute renal failure Thus Dayan et al (5) discovered in three patients that in the excited fibres taken from a suralis nerve biopsy specimen there were clear signs of segmental demyelinization although the time lag between the onset of clinical symptoms and biopsy was only 7–10 days It is therefore possible that the delayed deterioration of peripheral nerve functions in our patient 2 resulted from the subacute effect of uraemic toxins on the myelin sheath of the peripheral nerves Ten days after a single haemodialysis the patient was still rather azotaemic (Fig 1)

In the case of patients 1 and 2 (who before acute illness were not known to have any defect in the peripheral nerves) it is however difficult to believe that the demyelinization process was as rapid and transient as the initial changes in nerve function In these cases it seems more likely that the changes result from the immediate effect of the metabolic disturbance on the axon membrane and its conduction mechanism Also in patients 3–5 this is the most probable explanation although the possibility cannot be ruled out that the subclinical neuropathy probably connected with chronic uraemia has made the nerve fibres more prone to acute metabolic changes for example in such a way that more conduction blocks occur before dialysis than immediately after (6)

The change in nerve function cannot be due to temperature changes differences in temperature at the different times of measurement were insignificant and in addition temperature correction of individual NCV values was carried out (see methods) Subcutaneous oedema is however a critical factor when measuring NAPs with sk This holds good especially for the amplitude of NAP possibly also for its shape but hardly for its conduction time On the other hand there was no discernible oedema in any of our patients

The change in NAP amplitude and shape can however also be explained on the basis of selective blocking of nerve conduction caused by the metabolic disturbance It is known that the NAP amplitude and its rising phase derive from the thickest nerve fibres which have the highest conduction velocity (14) If the conduction block were affect the thickest nerve fibres selectively as has been supposed in the case of chronic azotaemia (6 this would lead to a fall in the amplitude of NAP and possibly a shortening of its rising phase Disappearance of the conduction block would thus explain the increase in NAP amplitude and rising time The validity of this hypothesis could perhaps be tested experimentally

It is possible to bring about significant NCV changes in animals through induced metabolic disturbance without simultaneous morphological changes taking place in the peripheral nerves (4) Inhalation anaesthetics are known to cause transient speeding up of NCV in man (22) On the other hand NCV is slowed reversibly for example by the effect of high serum Mg content (8) toxic di phenyl hydantoin concentration (3) and acute ischaemia (20) In these cases slowing of NCV is thought to be functional in character and the result of a decrease in Na K ATPase activity which maintains the polarization of the axon membrane As already pointed out Nielsen (17 18) has advanced the theory that some of the slowing down of NCV also in chronic uraemia may be due to the same mechanism

Theoretically all metabolic factors that have an effect on 1) the peak permeability of the axonal membrane 2) the membrane polarization level 3) the size of the membrane capacitor per unit area and 4) the resistivity of the cell plasma may cause changes in the conduction of action potentials of nerve fibres (25) What or which of these mechanisms are involved in acute uraemic intoxication and what or which changes of metabolite concentrations are most important can only by guessed at

It may be said that our results support Nielsen s theory (17 18) that uraemic intoxication causes functional and reversible slowing of NCV The mechanism must be distinguished from dis

in peripheral nerve function caused by morphological changes usually connected with terminal uraemia The practical conclusion to be drawn from this is that slight slowing down of NCV gives no assurance that there is polyneuropathy and that the degree of slowing down of NCV and its progression in successive measurements are conclusive A careful follow up study seems to be indicated in which a comparison would be made between the progression of clinical and metabolic symptoms on the one hand and neurophysiological symptoms on the other By means of such a study it might be possible to determine whether for example there are great interindividual variations in the slowing down of NCV in chronic uraemia patients thereby solving the important practical question of the critical degree of slowing down of NCV which will indicate with certainty that there is morphological damage to the nerves

ACKNOWLEDGEMENT

This investigation has been supported by the National Research Council for Medical Sciences Finland

REFERENCES

1 Asbury A K Uremic neuropathy In Peripheral neuropathy (ed P J Dyck P K Thomas & F H Lambert) vol II pp 982–992 Saunders Philadelphia 1975
2 Asbury A K Victor M & Adams R D Uremic polyneuropathy Arch Neurol 8 413 1963
Birket Smith E & Krogh E Motor nerve conduction velocity during diphenylhydantoin intoxication Acta Neurol Scand 47 265 1971
4 Bray G Dos P Aquayo A & Rasminsky M Nerve Na K ATPase activity in experimental diabetes PNC meeting Rochester (Minn) 1975
5 Dayan A D Gardner Thorpe C Down P F & *Gleadle R I* Peripheral neuropathy in uremia Neurology (Minneap) 20 649 1970
6 Dietz V & Freund H J Entladungsverhalten einzelner motorischer Einheiten bei uramischen Patienten J Neurol 207 255 1974
7 *Dinn J J & Crome D L* Schwann cell dysfunction in uremia J Neurol Neurosurg Psychiatry 33 605 1970
8 Fleming L W Lenman J A R & Stewart W K Effect of magnesium on nerve conduction velocity during regular dialysis treatment J Neurol Neurosurg Psychiatry 35 342 1972
9 Gilliatt R W Nerve conduction in human and experimental neuropathies Proc R Soc Med 59 98 1966
10 *Honet I C Jebsen R H* Tenckhoff H A McDonald J R Motor nerve conduction velocity chronic renal insufficiency Arch Phys Med 47 64 1966
11 Jebsen R H & Tenckhoff H A Comparison of motor and sensory nerve conduction velocity in early uremic polyneuropathy Arch Phys Med 50 1969
12 Jebsen R H Tenckhoff H & Honet J C Natural history of uremic polyneuropathy and effects of dialysis N Engl J Med 277 327 1967
13 Kemble F Electrodiagnosis of polyneuropathy Electromyography 7 187 1967
14 Lambert E H & Dyck P J Compound action potentials of sural nerve in vitro in peripheral neuropathy In Peripheral neuropathy (ed. P J Dyck P K Thomas & E H Lambert) vol I pp 427–441 Saunders Philadelphia 1975
15 Lang A H Forsstrom J Bjorkqvist S E & Kuusela V Statistical variation of nerve conduction velocity An analysis in normal subjects and uraemic patients J Neurol Sci 33 229 1977
16 Mattsson K Riska H Forsstrom J & Kock B Acute renal failure following rifampicin administration Scand J Respir Dis 55 291 1974
17 Nielsen V K The peripheral nerve function in chronic renal failure V Sensory and motor conduction velocity Acta Med Scand 194 445 1973
18 — The peripheral nerve function in chronic renal failure X Decremental nerve conduction in uremia Acta Med Scand 196 83 1974
19 — The peripheral nerve function in chronic renal failure Acta Med Scand (Suppl) 573 1974
20 Nielsen V K & Kardel T Decremental conduction in normal human nerves subjected to ischemia Acta Physiol Scand 92 249 1974
21 Preswick G & Jeremy D Subclinical polyneuropathy in renal insufficiency Lancet 2 731 1964
22 Rosner B S Clarc D C & Beck C Inhalation anesthetics and conduction velocity of human peripheral nerve Electroencephalogr Clin Neurophysiol 31 109 1971
23 Sharma A & Thomas P K Observations on peripheral nerve in rats following galactose feeding PNC meeting Rochester (Minn) 1975
24 Thomas P K Morphological basis for alterations in nerve conduction in peripheral neuropathy Proc R Soc Med 64 295 1971
25 Woodbury W Action potential Properties of excitable membranes In Physiology and biophysics (ed T C Ruch and H D Patton) p 1247 Saunders London 1965

Acta Med Scand 202 501–507 1977

Sodium Excretion and Blood Pressure of West and East Finns

M J Karvonen and S Punsar

From the Research Department Finnish Heart Association and the Department of Public Health Science University of Helsinki Helsinki Finland

ABSTRACT Altogether 1 178 men aged 55–74 were studied at 15 year re-examination of two rural cohorts of initially 1711 men from east and west Finland At the start, 15 years ago, the mean BPs were 148/89 and 140/82 mmHg in the eastern and western populations, respectively The difference had now disappeared and changed direction for systolic BP (east 147/85, west 155/84 mmHg) Of those with an initial systolic BP of 170 mmHg or higher, 68/118 (58%) in the east and 33/67 (49%) in the west had died in 15 years A 24-hour urine sample was collected for Na and K analysis from 20% of the men randomly selected Men in the east excreted less urine (1 64 vs 1 80 l/24 h, $p<0$ 05) but more Na than men in the west (245 vs 217 mEq/24 h, $p<0$ 05, 3 56 vs 2 92 mEq/kg b wt /24 h, $p<0$ 001) Regular medication with diuretics or antihypertensives did not affect the Na excretion The Na excretion showed a weak correlation with diastolic BP in the west ($r=-0$ 22, $p<0$ 05) but no correlation in the east The mean K excretion was approximately 90 mEq/24 h with no difference between east and west The estimated NaCl intake was in the east 16 0 in the 14 2 g/day According to international standards, the NaCl intake is high especially in the east, where male mortality from cardiovascular disease is highest in the world Energy expenditure and food intake are obvious determinants of Na excretion Ageing and incapacitation due to illness, including hypertensive disease, reduce food intake The observed negative correlation between BP and Na excretion can partly be ascribed to such factors partly to high mortality of individuals sensitive to damage caused by Na

In east Finland male cardiovascular mortality is twice as high as in the southwestern part of the country (18 27) When in 1959 two populations of men aged 40–59 years were examined in these areas the median systolic and diastolic BPs were substantially higher in the east 145/89 mmHg than in the west 137/81 mmHg (15) At later examinations in 1964 and 1969 this difference had however disappeared

In population comparisons the mean BP and sodium intake often show a positive correlation (23) The aim of this study was to investigate the relationship of BP to urinary sodium excretion within and between the two populations Previous information on sodium intake in east and west Finland was meagre (19) The 55–74 year old survivors of the two cohorts were invited to a 15 year follow up in 1974 A 24 hour urine sample was collected from every fifth man for determination of sodium excretion In analyzing the results the cardiovascular health record of each man was related to his sodium excretion and the two geographic areas were compared

STUDY POPULATION AND METHODS

The study was made in connection with the 15-year re examination of two cohorts of rural men aged 55–74 one from east the other from west Finland Originally in 1959 1711 men were drawn for a prospective study of coronary heart disease (CHD) (15 16) The total number of men re-examined was 1 178 (participation rate 96 2%) The examination was carried out in Sept –Oct 1974 Every fifth randomly chosen man from the populations was asked to collect a 24-hour urine sample The subjects were carefully instructed on the details of collection The samples were fetched from their homes and the subjects were interviewed on the completeness of the urine sampling Four samples were discarded as unreliable The urine samples were weighed and the results were transformed to volumes without correcting for specific weight Urinary sodium and potassium were determined by internal standard flame photometry (Flame Photometer 243 Instrumentation Laboratory)

Table I *Number age (mean) and weight (mean ± S E M) of men in the four medication groups of the two populations*

W=west E=east

	No of men		Age (y)		Weight (kg)	
	W	E	W	E	W	E
No diuretics or anti hypertensives (I)	98	94	63 7	62 9	74 1±1 2	69 7±1 4
Diuretics (II)	20	17	64 9	64 6	80 5±2 8	74 1±2 7
Antihypertensives (III)	10	5	62 3	59 4	71 5±4 2	63 8±2 8
Unidentified drug (IV)	6	1	64 0	63 0	76 3±4 9	84 0
Total	134	117	63 8	63 0	75 0±1 1	69 8±1 2

**$p<0\ 01$

Supine BP was measured by a physician at the end of an interview and physical examination using a mercury manometer Two readings were made to the nearest 2 mmHg and the means were used in the analyses (diastolic fifth phase) Two physicians performed the examinations each examining about one half of each geographic population

For studying the sodium and potassium excretions and their relationships to BP the two populations were divided into four groups according to current use of diuretics or antihypertensives I no medication II diuretics (with or without antihypertensives) III antihypertensives only IV unidentified drug used for hypertension All calculations were made separately in the four groups For most purposes however the results are given only for group I for the combined groups II IV and for the two pooled populations The medication groups are presented in Table I BP and associated findings in Table II Standard questionnaires and criteria were used in obtaining the clinical data in Table II

Differences were tested for significance with the *t* test e interrelationships were studied with correlation ysis For each analysis scatter diagrams were pre 'd to disclose any non linear associations The follow g significance limits were used in the statistical analyses *$p<0\ 05$ **$p<0\ 01$ ***$p<0\ 001$

RESULTS

The two populations were rather similar in the proportion of men receiving diuretics or other drugs that may affect the excretion of sodium and potassium and the BP and the mean age of men in the various groups was almost identical (Table I) The body weight of the men was lower in the east than in the west the difference was significant for the total populations and for men who were not on medication (group I) Men in the west also are approximately 3 cm taller than in the east (15) In the west subjects who were on diuretics (group II) were heavier ($p<0\ 05$) than those without medication other differences in weight within each population were not significant

In contrast to the situation in 1959 the mean systolic BP was now higher in the western than in the eastern cohort (Table II) There was no difference in the diastolic BP Regarding other characteristics listed in Table II, the two cohorts were rather similar

Urinary findings

The average 24 hour urine volume was slightly smaller but the concentration and output of sodium were higher in the eastern than in the western population (Table III) The differences in urine volume

Table II *Blood pressure (mmHg mean ± S E M) and associated findings in the two populations*

	West (N=134)		East (N=117)	
Systolic BP	158±2		148±2**	
Diastolic BP	84±1		84±1	
	N	%	N	%
Angina pectoris	25	19	31	26
Congestive heart failure	10	7	8	7
Stroke	7	5	3	3
Intermittent claudication	12	9	15	13
ECG (according to the Minnesota code (4))				
Infarction (1 1 2)	11	8	4	3
High QRS (3 1)	10	7	9	8
ST depression (4 1–3)	5	4	4	3

**$p<0\ 01$

[Tab]le III *24 hour urine volume and sodium excretion (mean±S E M) of men with no medication and of [tota]l populations*

[W=]west E=east

	N	24 hour urine volume (l)	Sodium concentration (mEq/l)	24 hour sodium excretion mEq	mEq/kg b wt
[No] diuretics or antihypertensives (group I)					
	98	1 79±0 06	129±5	218+ 8	2 97±0 10
	94	1 58±0 06*	160±5***	246±10*	3 58+0 14***
[Tota]l populations					
	134	1 80±0 05	128±4	217+ 7	2 92+0 09
	117	1 64±0 05*	155±4***	245± 9*	3 56±0 12***

[*p<]0 05 ***$p<0\ 001$

[Differences] in concentration and 24 hour excretion of [sod]ium between east and west were significant for [the] total populations as well as for groups with no [me]dication (group I) Within each population the [sod]ium excretion of those receiving diuretics or [ant]ihypertensives (groups II–IV not shown sepa[rate]ly in Table III) did not differ significantly from [tha]t of the others

[S]odium output correlated inversely with age in [the] eastern population ($r=-0\ 25$ $p<0\ 01$) but not [in t]he western ($r=0\ 00$) and positively with body [wei]ght in both populations (east $r-0\ 32$ $p<0\ 001$ [we]st $r=0\ 28$ $p<0\ 01$) The excretion of sodium was [the]refore calculated also as mEq/kg b wt (Table [III]) The transformed values showed a slightly [sma]ller variance and did not associate significantly [wit]h either age (east $r=-0\ 17$ west $r=0\ 01$) body [wei]ght (east $r=-0\ 18$ west $r=-0\ 15$) or treatment [gro]up

[T]he sodium output was on an average 28 mEq/24 [h h]igher in the eastern than in the western popula[tion] (Table III) This corresponds to a difference of [abo]ut 1 6 g in sodium chloride Per unit of body [wei]ght the men in the east excreted about 20% [mo]re sodium in the urine than the men in the west [Th]e 24 hour urinary excretion of potassium was [sim]ilar in the two populations less than half of that [of] sodium—east $x=92\pm3$ (S E M) mEq west [$x=$]90±2 (S E M) mEq

[Sod]ium excretion and blood pressure

[Th]e relationships within the populations between [BP] and urinary excretion of sodium were studied by [me]ans of correlation analyses using both the total [ex]cretion of sodium and the weight-corrected val[ues] as the independent variable A linear relation was assumed as there were no indications in scatter diagrams of another type of relationship over the range of BP levels Since (systolic) BP increased with age and since in the east sodium excretion decreased with age an attempt was made to elimi nate any confounding effect of age on the relation ships in a further correlation analysis actual data on BP and sodium excretion (per kg b wt) were replaced by the deviations of the individual values from their 5 year age and area specific means (Δ systolic and diastolic BP and Δ sodium mEq/kg b wt) The results of the three correlation analyses in the group with no medication (I) are shown in Table IV As seen the correlations between BP and sodium excretion were mostly negative some

Table IV *Correlations (r) of systolic (SBP) and diastolic (DBP) blood pressure (mean ± S E M) with 24 hour urinary excretion of sodium (a) and with the excretion of sodium/kg b wt (b) and the correlation of Δ SBP and Δ DBP with Δ sodium/kg b wt (c) in men without medication with diuretics or antihypertensives (group I)*

Δ–Difference between the actual value and the 5 year age and area specific mean
W=west E=east

	BP	r(a)	r(b)	r(c)
W (N=98)				
SBP	156±2	−0 16	−0 13	−0 11
DBP	84±1	−0 15	−0 21*	−0 22*
E (N=94)				
SBP	147±2	−0 03	−0 03	−0 04
DBP	84±1	0 10	−0 01	−0 07

*$p<0\ 05$

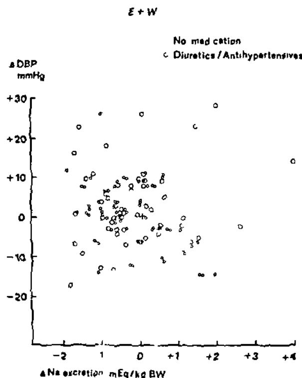


Fig 1 Relationship between diastolic blood pressure (*DBP*) and 24 hour urinary excretion of sodium in the pooled population Δ=Difference between the actual value and the 5 year age and area specific mean No medication (group I) $r=-0$ '4 diuretics and/or anti hypertensives (groups II–IV) $r=0$ 10 total $r=-0$ 09

significant in the west none in the east Similarly low were the correlations in the combined group of men on diuretics and/or antihypertensives (groups 'V) as well as in the total populations

,he relationships between diastolic BP and uri , sodium excretion are shown in Fig 1 in which differences between the actual values and their age and area specific means are used (Δ DBP and Δ Na/kg b wt) and the two populations are pooled Groups II–IV are presented separately from group I In both groups and in the total pooled population the correlations between Δ DBP and Δ Na excretion are non significant It should specifically be noted that there is no indication of opposite correlations at the two extremes of diastolic BP

DISCUSSION

Neither the determination of the 24 hour urinary excretion of Na as a measure of sodium intake nor the BP measurement are free from errors A correlation of $r=0$ 664 has been shown between 24 hour and 6-day urinary Na excretions while between 3- and 6 day collections it was 0 900 (22) Substanti losses of sweat may reduce the proportion c sodium excreted in urine However, this source c error was unlikely in the present population cor sidering the season the age of the men and their li habits

Assuming that 90% of the amount consumed i excreted in urine (9) the daily intake of NaCl in th west was 14 2 g and in the east 16 0 g These figure are evidently reliable and valid as present popula tion characteristics Their extrapolation to the pas remains a matter of conjecture Internationally th values are high though not extreme In Japan sal intakes of more than 25 g/day have been reporte (25, 28), recent data indicate a range from 8±3 t 20±7 g/day among five male populations with a mean age of 50 years (20 21) For Belgian men a mean 24 hour excretion of 11–12 g has beer published (13, 14) and in the USA the mean intak is reported to be approximately 10 g/day (24) Fo normotensive Swedish men, a mean excretion o 10 g NaCl (175 mEq Na)/24 h has recently bee reported (2)

The mean BP of the basic cohorts of the presen study varied at the five year repeat examinations a shown in Table V In 1959 the eastern cohort ha higher systolic and diastolic BP than the western At the 15 year re-examination the difference i systolic BP had become reversed and the diastoli BPs were identical The difference in systolic Bl was present also in men not receiving BP lowerin medication (Table IV) The eastern men excreted and evidently also consumed in their diet—mor sodium than the western men Thus the directio of the population difference in the 1959 BP readin was in line with the hypothesis of high salt intake a a cause of hypertension, while the 1974 readings fo systolic BP showed an opposite difference

Table V *Variations in mean blood pressur (mmHg) at 5 year intervals*

Year	West			East		
	SBP	DBP	*N*	SBP	DBP	*N*
1959	140	82	859	148	89	[illegible]
1964	139	81	801	140	82	[illegible]
1969	146	83	733	149	84	[illegible]
1974	155	84	625	147	85	[illegible]

The BP reading is subject to environmental physiological and psychological influences as well as to both systematic and random errors of measurement. Exposure to cold is one of the factors known to increase particularly the systolic BP. The five year examinations were always carried out in the east in September and in the southwest in October. As a rule there was little difference in the outdoor temperatures. However in 1974 the examination in the west was carried out in poorly heated premises and this might have increased the BP.

Another obvious source of variation is emotional tension. It is impossible to gauge its effects in the present study. If anything it might have contributed to the high pressure on the first occasion in the men in the east since they came from a poor sparsely populated area where contacts with outsiders were major events. Some degree of uncertainty has to be accepted in judging the BP readings also in the present study.

Whatever the BP readings may mean, the fact remains that in the east both mortality from cardiovascular diseases is higher and the prognosis of CHD worse than in the west (16, 26). These differences between east and west are rather large and they probably cannot be entirely attributed to the slightly higher concentrations of serum cholesterol and the more intense smoking habits in the east. The possibility of a toxic action of high sodium intake in addition to its hypertensive effect should also be considered. In a population whether it be rats or men some individuals are sensitive to the hypertensive action, others to the toxic action, some to both and some insensitive (1, 23). In a population eating a high salt diet those sensitive to both might die first, if those sensitive to only the toxic action die next, the remaining population would consist of aged normotensive and hypertensive individuals, both quite resistant to high salt intake. Any two populations like the east and west Finns might genetically consist of different proportions of sensitive individuals. However even with the same genetic background the population with the more intense exposure, here the east Finns, would eliminate the sensitive ones at an earlier age. In fact of those 118 men in the east who in 1959 had a systolic pressure of 170 mmHg or higher 68 (57.6%) had died by 1974. In the west the corresponding mortality had been 33 (49.3%) of 67 men.

High sodium intake and high BP associated with it are potent risk factors even in populations with little atherosclerosis. The five Japanese populations referred to above had been selected so that their salt and fat intake varied independently. The prevalence of hypertension and the incidence of cerebral apoplexy were largely parallel to salt intake while that of hypercholesterolemia, myocardial infarction and angina pectoris again were parallel to fat intake. The range of incidences was as wide as approximately tenfold both for cerebral apoplexy and CHD (20, 21). In Finland both serum cholesterol and sodium intake are high, particularly in the east.

While a positive correlation has been demonstrated between salt intake and BP at the population level, it has not been equally easy to show it between individuals within the same community. Joossens et al. (14) however found a correlation of $r=0.101$ ($p<0.0005$) between sodium excretion and systolic BP and of $r=0.114$ ($p<0.0001$) between sodium excretion and diastolic BP in 1314 men aged 17–85 years. The correlation coefficients were rather low. The sodium excretion was, as could be expected, correlated with stature and body weight.

In order to eliminate the effect of varying body size we expressed the sodium excretion also per kg b.wt. The age range of the subjects studied by Joossens et al. (14) was also wide and this must have resulted in a large variation in physical activity and food intake. Although the age range of our subjects was narrower, 20 years, we decided to standardize for age by dividing the series into 5 year age brackets and indicate the distance of each subject from his subgroup mean. Nevertheless no positive correlation between sodium intake and BP was observed among the individual subjects (Table IV).

In recent studies by Berglund et al. (2, 3) of normotensive and 19 hypertensive subjects the relation between sodium excretion and diastolic BP proved curvilinear: in normotensive subjects with diastolic BP up to 90 mmHg the 24 hour excretion of sodium rose ($r=0.54$) while sodium excretion fell at the level above 90 mmHg with rising pressure ($r=-0.55$). Another investigator (S. Ljungman) has confirmed this finding in a further series of 48 male hypertensives (11). Our data are at variance also with the above observations (Fig. 1), just as there was no systematic correlation in the entire series, so was none seen among those with high or low diastolic pressures separately. The mean diastolic BP in the present series was 84 mmHg and re-

ably near to the claimed turning point 90 mmHg Our observations thus cover approximately the same range as those of Berglund et al (3) They interpret their findings as being due to renal mechanisms

In a steady state the urinary excretion sodium closely reflects the amount eaten day day the biological half life of sodium in ma s only 1–2 weeks (12) Among subjects eatir similar food those who spend less energy al eat—and ex crete—less salt a correlation coefficient of $r=0\ 524$ was found between daily calorie d sodium intakes (19) Seriously ill people as a roup spend less energy than people in good health Hypertensive patients particularly those with a decompensated heart are ill people in the es of Berglund et al (3) 10 patients were classi 1 is belonging to WHO stage 1 6 to stage 2 and stage 3 (1 not speci fied) We ventu to t st th the decrease in sodium excre in wi re s g diastolic BP among patients h hyp sion is ssentially due to their physic incap o an consequently reduced food upt ke k to give any direct clues on renal fu akness of the corresponding correlat r s (Table IV) may be due to th n lor sample of the total population an enriched inclusion of patie

In a clinical t orthiazide in 16 patients with WH pertension (8) the urinary 24 hour ex dium had increased after 3 months of the i was concluded in that dy that protr tr atment leads to increased intake Our s es cluded 37 patients on tics and altogether 59 patients medicated with lowering agents The mean sodium excretion of the patients on medication did not differ from that of men with no medication According to our results, medication with diuretics or antihypertensives does not affect the salt consumption

Both epidemiological and experimental studies have for decades contributed data to suggest high sodium intake as a causative factor in hypertension (13 23 29) In the 1940s Kempner's low sodium diet was successfully used for reducing BP of hypertensive patients (17 29) A recent study in an animal model (7) has demonstrated how sodium intake and nervous stress may interact in causing hypertension Brain receptors sensitive to sodium concentration in the cerebrospinal fluid are able to induce a rise of BP in the conscious animal (5) Several observations have been unified to a theory which connects the underlying physiological and pathogenetic mechanisms (6 10) It is hardly probable that further essential information on the causation of hypertension could be obtained with purely observational and analytical methods A long term intervention study appears to be needed to demonstrate whether the incidence of hypertension in a population with high sodium intake could be prevented by decreasing the sodium intake (and/or by increasing the potassium intake (23)) We consider the time opportune for such a study

ACKNOWLEDGEMENTS

The study was supported by grants from the Finnish Academy the Operation Red Heart Foundation of the Finnish Lions Organization and Oy Hartwall Ab Manufacturers Helsinki Finland

REFERENCES

1 Aoki K Yamori Y Ooshima A & Okamoto K Effects of high or low sodium intake in spontaneously hypertensive rats Jpn Circ J 36 539 1972
2 Berglund G Aurell M & Wilhelmsen L Renal function in normo- and hypertensive 50-year-old males Acta Med Scand 199 25 1976
3 Berglund G Wikstrand J Wallentin I & Wilhelmsen L Sodium excretion and sympathetic activity in relation to severity of hypertensive disease Lancet 1 324 1976
4 Blackburn H Keys A Simonson E Rautaharju P & Punsar S The electrocardiogram in population studies A classification system Circulation 21 1160 1960
5 Eriksson L & Fyhrquist F Plasma renin activity following central infusion of angiotensin II and altered CSF sodium concentration in the conscious goat Acta Physiol Scand 98 209 1976
6 Freis E D Salt volume and the prevention of hypertension Circulation 53 589 1976
7 Friedman R & Iwai J Genetic predisposition and stress induced hypertension Science 193 161 1976
8 Fyhrquist F & Kurppa K Blodtryck salthunger och renin vid behandling med hydroklortiazid In Hypertonisymposium Saltsjobaden 21–22 Sept 1974 pp 187–189 Bayer Uppsala 1975
9 Geigy scientific tables 6th ed Geigy Basle 1967
10 Guyton A C Coleman T G Cowley A W Jr Scheel K W Manning R D & Norman R A Arterial pressure regulation overriding dominance of the kidneys in long term regulation and in hypertension Am J Med 52 584 1972
11 Hansson L Marstrandsmötet 1976 Aktuell hypertoniforskning i Norden Lakartidningen 73 3516 1976
12 Hasänen E & Rahola T The biological half life of ^{137}Cs and ^{24}Na in man Ann Clin Res 3 236 1971

13 Joossens J V Salt and hypertension water hardness and cardiovascular death rate Triangle 12 9 1973
14 Joossens J V Willems J Claessens J Claes J & Lissens W Sodium and hypertension In Nutrition and cardiovascular diseases (ed F Fidanza A Keys G Ricci & J C Somogyi) pp 91-110 Morgagni Edizioni Scientifiche Rome 1971
15 Karvonen M J Blomqvist G Kallio V Orma E *Punsar S Rautaharju P Takkunen J & Keys A* Men in rural East and West Finland In Epidemiological studies related to coronary heart disease characteristics of men aged 40-59 in seven countries (ed A Keys et al) Acta Med Scand (Suppl) 460 169 374 1967
16 Karvonen M J Orma E Punsar S Kallio V Arstila M Luomanmäki K & Takkunen J Five year experience in Finland In Coronary heart disease in seven countries (ed A Keys) Am Heart Assoc Monogr 29 59 1970 Also in Circulation (Suppl) *1 1970*
17 Kempner W Treatment of hypertensive vascular disease with rice diet Am J Med 4 545 1948
18 Keys A Karvonen M J & Fidanza F Serum cholesterol studies in Finland Lancet 2 175 1958
19 Koivistoinen P Ahlström A Nissinen H Pekkarinen M & Roine P Mineral element compositions of Finnish diets Part I Fe Cu Mn Zn Mg Na K Ca and P Suom Kemistilehti B 43 426 1970
20 Komachi Y Iida M Ozawa H Shimamoto T Chikayama Y Takahashi H Konishi M & Ueshima H Comparison of risk factors of CHD and CVA in several groups in Japan with special reference to dietary intake In Physiological adaptability and nutritional status of the Japanese (ed K Asahina & R Shigiya) B Growth work capacity and nutrition of Japanese JIBP synthesis vol 4 Human adaptability vol 3 pp 239-246 University of Tokyo Press Tokyo 1975
21 Komachi Y Iida M Shimamoto T Chikayama Y Takahashi H Konishi M & Tominaga S Geographic and occupational comparisons of risk factors in cardiovascular diseases in Japan Jpn Circ J *35 189 1971*
22 Langford H & Watson R L Electrolytes and hypertension In Epidemiology and control of hypertension (ed O Paul) pp 119-130 Stratton Intercontinental Medical Book Corp Chicago 1975
23 Meneely G R & Batterbee H D High sodium-low potassium environment and hypertension Am J Cardiol 38 768 1976
24 Meneely G R & Dahl L K Electrolytes in hypertension the effects of sodium chloride The evidence from animal and human studies Hypertension *and its treatment Med Clin North Am 45 271 1961*
25 Ozawa H Cited in Komachi et al (21)
26 Punsar S & Karvonen M J Angina pectoris and ECG abnormalities in relation to prognosis of coronary heart disease in population studies in Finland In Early diagnosis of coronary heart disease (ed P Halonen & A Louhija) Adv Cardiol 8 148 1973
27 Puska P Regional differences in cardiovascular death rates (in Finnish) Suom Lääk Lehti 27 3071 1972
28 Sasaki N The relationship of salt intake to hypertension in the Japanese Geriatrics 19 735 1964
29 Weinsier R L Overview salt and the development of essential hypertension Prev Med 5 7 1976

Acta Med Scand 202 509–516 1977

Plasma Renin, Plasma Aldosterone and Exchangea 'e Sodiu 1 in Normotensive and Hypertensive Kidney Transplan Recipi with and without Transplant Renal Artery Steno s

H J Kornerup and E B Pedersen

From Department of Medicine C Århus Kommunehospital and University of Århus Den ,

BSTRACT Blood pressure (BP), plasma renin oncentration (PRC), plasma aldosterone concen ration (PAC) and exchangeable sodium (ES) were tudied *in 19* kidney recipients on different fixed evels of sodium intake after *successful kidney transplantation* The following groups of kidney recipients ere investigated group 1 7 normotensives group 7 hypertensives without transplant renal artery tenosis (TRAS), group 3 5 hypertensives with an iographically verified TRAS Hypertension in the ecipients without TRAS (group 2) was characterized y a positive correlation between BP and ES and a ormal response of PRC and PAC to a fixed low (10 Eq/day) and high (150 mEq/day) sodium intake In ontrast, hypertension in the recipients with TRAS group 3) was characterized by a normal or varyingly creased PRC on a liberal sodium intake and a educed response of PRC to sodium restriction, hereas PAC did not differ from the other groups of ecipients In one recipient in group 3 who underwent urgical correction for TRAS, PRC and PAC dereased before operation during sodium restriction, ut BP remained high until after operation when it ormalized simultaneously with a decrease in ES he results indicate that sodium retention is involved the pathogenesis of posttransplant hypertension nd suggest that an increased activity of the re in–angiotensin system is counterbalanced by an ac umulation of sodium in TRAS

The significance of the renin–aldosterone system nd sodium and water balance in hypertension fol owing kidney transplantation is still obscure In cute allograft rejection the renin content of plasma s usually increased (10 14 20 22 27 29) whereas it is usually normal when kidney funct on is st (2 18 20 23) In the few previous reports of post transplant hypertension with transplant renal artery stenosis (TRAS) renin release was normal in the study by Grunfeld et al (9) whereas Bennett et al (1) Margules et al (15) and Wallenius et al (28) found an increased plasma renin content in TRAS Sampson et al (23) have postulated that posttrans plant hypertension is caused by aldosterone excess This statement could not be confirmed in the study by Bennett et al (1) No information is available concerning the role of body sodium content in post transplant hypertension

The purpose of the present study was to elucidate the significance of the renin–aldosterone system and sodium balance in hypertension following kid ney transplantation by studying the relationship between blood pressure (BP) plasma renin con centration (PRC) plasma aldosterone concentra tion (PAC) and exchangeable sodium (ES) in nor motensive and hypertensive kidney transplant re cipients with and without TRAS In order to reduce the variables affecting the renin–aldosterone sys tem the investigations were limited to a homogeneous group of recipients with stable kidney function several months after successful kidney transplantation and without evidence of allograft reaction Bilateral nephrectomy had been per formed in all recipients before the time of the study sodium intake was fixed at different levels during the study antihypertensive agents and/or diuretics were discontinued several weeks before the study and the dosages of prednisone used for immunosuppression were low

Table I *Clinical data on the patients investigated*

Pat no	Age (y)	Sex	Time after transplantation (mo)	BP (mmHg)	Creatinine clearance (ml/min)	Prednisone (mg/d)	Azathioprine (mg/d)	Hypertension before transplantation
Group 1								
1	50	♂	49	122/83	82	7 5	100	+
2	53	♂	75	128/83	74	0 0	175	+
3	46	♀	51	107/75	47	5 0	100	+
4	39	♀	94	107/78	132	2 5	125	+
5	49	♀	108	122/73	97	1 25	100	0
6	47	♀	7	120/80	50	17 5	75	+
7	57	♀	22	138/80	92	10 0	125	+
Group 2								
8	26	♂	7	172/112	40	17 5	100	0
9	38	♀	50	135/103	50	15 0	100	0
10	29	♂	101	138/105	88	1 25	150	+
11	64	♀	15	187/120	93	10 0	125	0
12	36	♂	12	212/105	49	10 0	125	+
13	58	♀	13	185/117	57	10 0	75	+
14	33	♂	31	160/100	65	10 0	125	+
Group 3								
15	37	♀	22	167/107	92	5 0	150	+
16	55	♀	30	177/115	136	5 0	125	+
17	54	♀	64	160/103	89	5 0	25	0
18	42	♀	25	168/107	67	10 0	100	+
19	39	♀	52	210/120	54	5 0	100	+

SUBJECTS

Nineteen kidney transplant recipients 13 females and 6 es aged 26–64 years (mean (±1 S D) 44 8±10 5) *tudied 7–108* months (mean (±1 S D) 43 6±32 2) transplantation Sixteen had received a cadav 3 a *related living-donor* kidney The primary disease in 7 recipients was chronic pyelonephritis *chronic glomerulonephritis* in 3 polycystic disease 2 hereditary nephritis (Alport) and in one patient each *diabetic nephropathy* congenital renal hypoplasia renal tuberculosis and renal vascular occlusion At the time of the study bilateral nephrectomy had been performed in *all recipients*

Clinical data are given in Table I At the time of the study the function of the kidney graft was stable 24-hour creatinine clearance being 40–136 ml/min (mean *(±1 S D) 76 5±27 5)* Proteinuria was absent in all except one patient (no 12) who had a proteinuria of 2 g/day The immunosuppressive treatment included prednisone 1 25–17 5 mg/day (mean (±1 S D) 7 8±5 2) and azathioprine 25–175 mg/day (mean (±1 S D) 110 5±32 6) Obstructive nephropathy in the kidney graft was excluded by urography in all Of the 9 hypertensive recipients studied transplant renal arteriography demonstrated TRAS i e reduction of more than 50% of the main artery in 5 In three of these patients with TRAS an arterial bruit was heard over the graft.

The recipients were divided into three groups on the basis of the average BP measured at the last three consecutive visits to the Out Patient Clinic Group 1 Seven normotensive recipients (nos 1–7) Their BP averaged 121/79±11/4 mmHg (1 S D) and no hypertensive l were apparent Group 2 Seven hypertensive reci (nos 8–14) i e diastolic BP≥100 mmHg without T Their BP averaged 170/109±28/8 mmHg (1 S D) A hypertensive retinal changes of grade I–II (Keith ner) One recipient (no 12) had diabetic prolif retinopathy On X ray 2 recipients had an increase dio/thoracic ratio and 3 had hypertrophy and/or str ECG Group 3 Five hypertensive recipients (nos with angiographically verified TRAS Their BP av 176/110±20/6 mmHg (1 S D) All had hypertensive changes of grade I–II (Keith Wagener) Two reci had an increased cardio/thoracic ratio on chest and 3 had ECG hypertrophy and/or strain

Hypertension developed during the first 3 month transplantation in 9 recipients (4 with and 5 w TRAS) and more than 6 months after transplanta 3 (1 with and 2 without TRAS) There were no sign differences between the groups in terms of age tension before transplantation months after transp tion creatinine clearance or dosages of predniso azathioprine ($p>0$ 05)

The following groups of normal controls were st 1) PRC was measured in 15 normals 4 females a males (mean age (±1 S D) 27 8±9 8 years) 2) PA measured in 12 normals 6 females and 6 males age (±1 S D) 48 6±20 1 years) 3) ES was measu 16 normals 8 females and 8 males (mean age (± 49 2±14 7 years) The nature of the study was exp to all patients and controls before the examination consent was obtained from all

Procedure

Antihypertensive treatment and/or diuretics were dis ontinued at least 4 weeks before the study which was erformed during a hospital stay of 12 days In hospital he patients received a diet with a fixed sodium content f 10 mEq/24 hours for the whole experimental period uring the last 6 days the diet was supplemented with odium 150 mEq/24 hours as sodium chloride tablets lood specimens for determination of PRC and PAC were ken at 8–9 a m after bedrest and an 8 hour fast at the art of the study and at the end of each of the two etary periods ES was measured at the end of each of e two dietary periods Mean BP (diastolic BP+1/3 BP nplitude) calculated from the average of three measure ents of the supine BP at the end of the two dietary riods was employed for the statistical calculations ie controls were studied without dietary restrictions

METHODS

asma renin concentration was measured using the ethod described by Giese et al (6) This involves radio-imunoassay of angiotensin I after incubation of dialysed asma at 37°C and pH 7 4 with and without addition of internal standard of human renin and extraction of the giotensin I produced The Medical Research Council vision of Biological Standards National Institute for edical Research Mill Hill London supplied us with enin Human 68/356 Dr J Giese et al Department of inical Physiology Glostrup Hospital Copenhagen enmark placed antiserum against angiotensin I at our sposal PRC are given in micro Goldblatt Units per ml asma (μGU/ml) using the above mentioned standard iman renin as reference The coefficient of variation for e reproducibility of the analysis (day to day) was 11% ithin the range 10–175 μGU/ml

Plasma aldosterone concentration was measured using e method described by Rask Madsen et al (21) This is radioimmunologic measurement performed on plasma ter previous extraction with dichloromethan purifica on on silica gel columns and chromatographic separa on on paper The position of aldosterone on the paper rips was located by scanning Antiserum against aldo-erone was obtained from Research Plus Laboratories enville New Jersey The coefficient of variation for the eproducibility of the analysis (day to day) was 15% with the range 2 6–25 9 ng/100 ml

Exchangeable sodium was measured as 24 hours ex hangeable ^{24}Na using an isotope dilution technique as escribed by Hansen (11) The regression line for the rela onship between ES (mEq/kg) and leanness index height^3/weight) in normal controls ($y=341\ 9x+15\ 8$ $=16$ $r=0\ 89$ $p<0\ 001$) was used as reference ES values ere expressed as a percentage of expected ES in nor nals with the same leanness index (4)

Statistics Wilcoxon s signed rank test and Mann Whitney s rank sum test were used for paired and un aired comparison within groups and between groups espectively Spearman s test was used for estimating cor elations Valu

RESULTS

Results in the three groups of kidney recipients are given in Tables II and III

Group I (normotensive recipients)

On liberal sodium intake PRC and PAC were nor mal in all but one recipient in group I compared with the non transplanted control groups the ex ception (no 7) having a slightly elevated PRC of 88 μGU/ml No differences in PRC and PAC were present between group I 50±26 μGU/ml and 8 5± 2 6 ng/100 ml respectively and the normal controls 36±16 μGU/ml and 11 5±4 2 ng/100 ml respective ly ($p>0\ 10$) On a fixed low sodium intake urinary sodium excretion decreased to 17±11 mEq/day Simultaneously PRC increased from 50±26 to 76± 22 μGU/ml and PAC from 8 5±2 6 to 17 2±5 4 ng/100 ml in all recipients On a fixed high sodium intake urinary sodium excretion increased to 159±

Table II *PRC and PAC on liberal (A) low (10 mEq/day) (B) and high sodium intake (150 mEq/day) (C)*

Pat no	PRC (μGU/ml) A	B	C	PAC (ng/100 ml) A	B	C
Group 1						
1	49	79	33	7 7	11 6	4 4
2	18	94	18	9 0	19 3	5 8
3	–	48	15	–	–	–
4	73	81	44	6 0	13 5	5 2
5	39	63	39	–	–	–
6	34	56	38	7 0	25 4	2 0
7	88	109	34	12 6	16 3	5 8
Mean	50	76	32	8 5	17 2	4 6
S D	26	22	11	2 6	5 4	1 6
Group 2						
8	51	103	26	–	–	–
9	22	50	23	2 0	4 5	2 0
10	34	63	21	10 8	15 6	5 2
11	20	31	17	–	–	–
12	66	74	41	16 3	29 2	9 2
13	25	36	29	10 1	25 5	15 3
14	40	61	31	5 9	9 3	3 0
Mean	37	60	27	9 0	16 8	6 9
S D	17	24	8	5 4	10 5	5 4
Group 3						
15	51	78	68	5 1	7 5	4 8
16	131	120	29	18 2	41 4	7 6
17	10	26	7	4 9	11 6	3 7
18	110	116	63	7 8	11 4	2 7
19	340	21	–	86 9	6 6	–
Mean	128	72	42	24 6	15 7	4 7
S D	128	47	29	35 3		

Table III *BP ES urinary sodium excretion body weight height and leanness index on low (10 m... day) (A) and high sodium intake (150 mEq/day) (B)*

Pat no	BP (mmHg)		ES (%)		Urinary sodium excr (mEq/d)		Body weight (kg)	Height (cm)	Leann index (m³/kg
	A	B	A	B	A	B			
Group 1									
1	110/65	110/75	99 6	108 9	36	139	51 0	171	0 098
2	131/90	119/85	96 5	107 6	12	141	80 6	178	0 070
3	113/73	113/70	97 9	102 9	11	180	42 9	159	0 094
4	104/73	101/70	104 8	110 2	2	130	64 0	164	0 069
5	105/70	110/70	116 7	116 4	17	197	38 5	148	0 084
6	116/78	111/78	106 9	108 2	16	189	43 2	148	0 075
7	123/68	121/70	100 8	106 6	22	140	61 9	163	0 070
Mean	115/74	112/74	103 3	108 7	17	159	54 6	162	0 080
S D	10/8	7/6	7 0	4 1	11	28	15 0	11	0 017
Group 2									
8	165/110	160/110	90 0	93 4	45	136	65 0	170	0 076
9	124/100	127/100	87 4	89 9	17	165	59 6	169	0 081
10	149/110	152/114	87 3	97 4	5	87	70 0	175	0 077
11	190/108	185/108	92 1	99 4	39	184	89 8	173	0 058
12	178/100	190/105	98 7	108 3	24	169	60 9	177	0 091
13	190/130	211/130	115 0	121 6	45	152	65 8	160	0 067
14	155/100	181/111	89 1	99 1	25	122	70 0	178	0 081
Mean	164/108	172/111	94 2	101 3	29	145	68 7	172	0 075
S D	24/11	28/9	10 0	10 6	15	33	10 1	6	0 012
Group 3									
15	163/105	135/95	77 3	81 9	7	160	91 6	173	0 057
16	170/110	150/100	78 6	–	5	221	72 7	162	0 059
17	162/111	155/113	100 3	106 6	34	148	66 4	169	0 073
18	183/100	190/105	99 8	119 0	47	174	44 0	157	0 088
9	208/120	–	96 9	–	28	–	58 6	176	0 093
ean	177/109	158/103	90 6	102 5	24	176	66 7	167	0 074
D	19/7	23/8	11 6	18 9	18	32	17 6	8	0 016

28 mEq/day Simultaneously PRC decreased from 76±22 to 32±11 μGU/ml and PAC from 17 2±5 4 to 4 6±1 6 ng/100 ml in all recipients

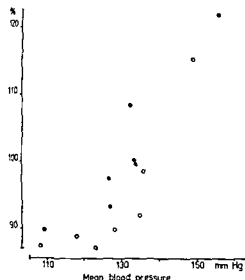
ES was normal in all recipients in group 1 since their values on both sodium regimes were very similar to those in the normal controls with the same leanness index

Group 2 (hypertensive recipients without TRAS)

On a liberal sodium intake PRC (37±17 μGU/ml) and PAC (9 0±5 4 ng/100 ml) were normal in all recipients in group 2 During sodium restriction urinary sodium excretion decreased to 29±15 mEq/day Simultaneously PRC increased from 37±17 to 60±24 μGU/ml and PAC from 9 0±5 4 to 16 8±10 5 ng/100 ml in all recipients During oral sodium supply, urinary sodium excretion increased to 145±33 mEq/day Simultaneously PRC decreased fr... 60±24 to 27±8 μGU/ml and PAC from 16 8±10.5... 6 9±5 4 ng/100 ml in all recipients No differenc... in PRC and PAC between the recipients in groups... and 1 were present on different sodium regim... (p>0 10)

A positive correlation was found between me... BP and ES on both sodium regimes (rho=0 7... 0 88 n=7 p<0 05) which is illustrated in Fig... Mean BP and ES were not correlated to dosag... of prednisone (p>0 10)

On a fixed low sodium intake ES in group... (94 2%±10 0) was significantly decreased co... pared with group 1 (103 3%±7 0) (p<0 05) but... a fixed high sodium intake the difference betwe... the two groups of recipients was not significa... (p>0 10)



g 1 Relationship between mean BP and exchangeable dium in hypertensive recipients without transplant renal tery stenosis (group 2) on fixed low (O) and high sodi n intake (●)

oup 3 (hypertensive recipients with TRAS)

great variation in PRC was present in group 3 on liberal sodium intake Thus in one recipient (no) PRC was low 10 μGU/ml in one (no 15) it as normal 51 μGU/ml and increased in three os 16 18 19) ranging from 110 to 340 μGU/ml n a liberal sodium intake PAC was normal in all cipients in group 3 except one (no 19) who coin dent with a greatly increased PRC (340 μGU/ml) so had a greatly increased PAC (86 9 ng/100 ml) In a fixed low sodium intake urinary sodium ex etion decreased to 24±18 mEq/day The response PRC to sodium restriction varied greatly Thus uring sodium restriction PRC decreased paradoxi ally in one recipient (no 19) from 340 to 21 μGU/ l in two (nos 16 18) it did not change and in reased slightly in two (nos 15 17) PAC increased uring sodium restriction in all patients except one no 19) in whom PAC decreased parallel to a de rease in PRC On a fixed high sodium intake, rinary sodium excretion increased to 176±32 nEq/day Simultaneously PRC decreased from 72± 7 to 42±29 μGU/ml and PAC from 15 7±14 5 to 17±2 1 ng/100 ml in all recipients No differences n PRC and PAC between the groups of recipients were present on low or high sodium regimes $p>0$ 10)

A positive correlation between PRC and PAC was found in group 3 on a liberal sodium intake (rho=1 00, $n=5$ $p<0$ 05) but not on a fixed low or high sodium intake

ES in group 3 was very similar to that in group 2 on both sodium regimes and did not differ signifi cantly from ES in group 1 ($p>0$ 10) No correlation was found between mean BP and ES

Surgical correction for TRAS was performed in one recipient in group 3 (no 19) The course in this patient is illustrated in Fig 2 which shows that PRC and PAC decreased before operation but BP remained high until after operation when it nor malized simultaneously with a decrease in ES

DISCUSSION

The results of the present study demonstrate that an abnormal sodium balance as well as an abnormal activity of the renin–angiotensin system may be in volved in the pathogenesis of hypertension follow ing kidney transplantation Thus hypertension in the recipients without TRAS (group 2) was char acterized by a positive correlation between BP and ES and by a normal response of the renin–

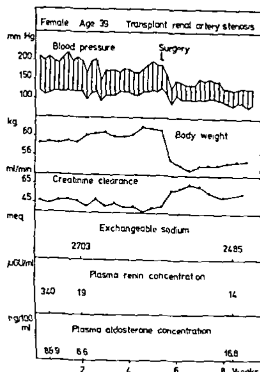


Fig 2 Relationship between BP plasma renin concentra tion plasma aldosterone concentration and exchangeable sodium in a recipient operated on for transplant renal

angiotensin system to different sodium intakes whereas hypertension in the recipients with TRAS (group 3) was characterized by a normal or varying ly increased PRC and a reduced response of PRC to sodium restriction In one patient in group 3, BP normalized simultaneously with a reduction of ES following surgical correction of TRAS

In previous studies of the renin–angiotensin sys tem in patients with stable renal function after kid ney transplantation the basal plasma renin content and the response to different sodium regimes have unanimously been reported as being normal (3 7 12) However, in these previous studies the ability of the transplanted kidney to respond normally to different sodium intakes has not been related to posttransplant hypertension In the hypertensives without TRAS in the present study (group 2) we found a normal PRC on a liberal sodium intake and PRC increased during sodium restriction and de creased during sodium supply without differences in the pattern of PRC between the normotensives (group 1) and the hypertensives (group 2)

In contrast to those hypertensive recipients with out TRAS of the 5 hypertensives with TRAS (group 3) 3 had a varyingly increased PRC on a liberal sodium intake This is in accordance with the few previous studies of the renin–angiotensin system in patients with TRAS (1 9 15, 28) Margules et al (15) found increased peripheral plasma renin activity (PRA) and angiotensin II in both the 2 patients investigated Bennett et al (1) found in creased PRA in the graft vein in 2 of 4 patients and Wallenius et al (28) found increased peripheral PRA in 4 of 7 patients whereas Grunfeld et al (9) found increased renin release from the graft in only 2 of 10 patients with TRAS Thus in the previous studies on TRAS evidence for an increased renin release from the graft was present in approximately 40% but with a great variation

In the study by Bennett et al (1) PRA in the graft vein was higher in the hypertensive patients with stenosis of the main artery of the kidney trans plant or narrowing of the intrarenal arteries caused by angiographically suspected chronic vascular allograft reaction compared with normotensive patients when a fixed low sodium intake (10 mEq/ day) was employed whereas no differences in PRA between normotensives and hypertensives were present on a fixed high sodium intake (200 mEq/ day) In contrast to their study, sodium restriction in our study resulted in a reduced response in renin release in the recipients with TRAS (group 3) rats with one kidney Goldblatt hypertension it possible to convert the predominantly salt water dependent hypertension to a renin-depend form of hypertension by sodium depletion (Therefore it is possible that a more vigoro sodium depletion in our patients would contrib to a better separation between the patient groups

Sampson et al (23) found an increased ald sterone secretion rate in patients with hypertens after kidney transplantation suggesting that po transplant hypertension may be caused by mine alocorticoid excess This was not confirmed in t present study since PAC was normal on a libe sodium intake and a normal response of PAC varying sodium intakes appeared in all except recipient with TRAS Hyperaldosteronism in th patient probably was secondary to an increas renin release as PAC changed parallel to PR during sodium restriction Furthermore PAC co related positively to PRC in the group of reci ents with TRAS (group 3) when on liberal sod intake

Previous studies provide no information on t significance of the body sodium content for po transplant hypertension In this study we found positive correlation between BP and ES in t hypertensive recipients without TRAS (group indicating that sodium retention is involved in t pathogenesis of posttransplant hypertension wi out TRAS

An inherent problem in using ES as a measur ment of body sodium content is the absence of ideal reference for expressing ES values Th body weight is not useful for this purpose beca the low sodium content of adipose tissue results lower ES in overweight individuals In order reduce the influence of different degrees of a positas in the recipients studied we have used leanness index for expressing ES values as commended by others (4) However even a "lea ness index as reference for ES may not be s ficient to eliminate the influence of different degr of adipositas This may explain why ES was sig icantly lower in the hypertensives without TR than in the normotensives when on a fixed l sodium intake since body weight was higher in t former

The patients in this study had one kidney— kidney graft—because they had all undergone lateral nephrectomy Therefore hypertension

e recipients with TRAS is analogous to experi ental hypertension in animals produced by clip ng one renal artery and removing the contralateral dney i e one kidney Goldblatt hypertension his type of hypertension in rats is characterized an initial, transitory increase in plasma renin llowed by a normalization of this and sodium re ntion (8 26) Conversely unclipping of the renal tery in one kidney Goldblatt hypertension results a massive natriuresis and normalization of BP 3) *In our patients with TRAS* (group 3) ES lues were very similar to those in the hyperten ve patients without TRAS (group 2) However e course of the patient who underwent surgical rrection for TRAS was an illustrative example of e relationship between renin release and sodium lance indicating that increased activity of the nin–angiotensin system is counterbalanced by dium and fluid retention in the human one kidney odel analogous to the corresponding animal odel

Successful surgical correction of TRAS has pre ously been reported (1 9 15 16 17 19 24 25) However in only a few of these studies was asma renin investigated (1 9 15 28) In the study Wallenius et al (28) PRA was increased in 4 pa nts with TRAS who benefited from operation hereas BP was unchanged after operation in 2 tients with normal PRA Bennett et al (1) have ported an increased PRA in the graft vein in 2 pa nts operated on for TRAS and Margules et al 5) have also reported an increased peripheral RA or angiotensin II level in 2 patients operated for TRAS This contrasts with the study of runfeld et al (9) who found a normal renin se etion rate in 3 of 4 patients cured of hypertension surgical correction of TRAS Thus the predic e value of the plasma renin with regard to the ect of surgical correction of TRAS on hyperten on is obscure which is also illustrated by the pa nt in this study who underwent operation for RAS Therefore the indication for surgical cor ction of TRAS has to be based upon an evalua n of the severity of hypertension and degree of tery stenosis until more evidence is available on e predictive value of plasma renin determinations

ACKNOWLEDGEMENTS

he study was supported by grants from Statens læge denskabelige Forskningsråd Hjerteforeningen F L hmidt s Fond and Cai Hol

REFERENCES

1 Bennett W M McDonald W J Lawson R K & Porter G A Posttransplant hypertension Studies of cortical blood flow and the renal pressor system Kidney Int 6 99 1974

2 Blaufox M D Birbari A E Hickler R B & Merrill J P Peripheral plasma renin activity in renal homotransplant recipients N Engl J Med 275 1165 1966

3 Blaufox M D Lewis E J Jagger P Lauler D Hickler R & Merrill J P Physiologic responses of the transplanted human kidney Sodium regulation and renin secretion N Engl J Med 280 62 1969

4 Davies D L Beevers D G Briggs J D Medina A M Robertson J I S Shalekamp M A Brown J J Lever A F Morton J J & Tree M Abnormal relation between exchangeable sodium and the renin–angiotensin system in malignant hyper tension and in hypertension with chronic renal fail ure Lancet 1 683 1973

5 Gavras H Brunner H R Vaughan E D Jr & Laragh J H Angiotensin–sodium interaction in blood pressure maintenance of renal hypertensive and normotensive rats Science 180 1369 1973

6 Giese J Jørgensen M Nielsen M D Lund J O & Munck O Plasma renin concentration measured by use of radioimmunoassay for angiotensin I Scand J Clin Lab Invest 26 355 1970

7 Green J A Vander A J & Kowalczyk R S Plasma renin activity and aldosterone excretion after renal homotransplantation J Lab Clin Med 71 586 1968

8 Gross F The renin–angiotensin system and hyper tension Ann Intern Med 75 777 1971

9 Grunfeld J P Kleinknecht D Moreau J F Ka moun P Sabto J Garcia Torres R Osorio M & Kreis H Permanent hypertension after renal homo- transplantation in man Clin Sci Mol Med 48 391 1975

10 Gunnells J Stickel D L & Robinson R R Episodic hypertension associated with positive renin assay after renal transplantation N Engl J Med 274 543 1966

11 Hansen J Hydrochlorothiazide in the treatment of hypertension The effects on the blood volume ex changeable sodium and blood pressure Acta Med Scand 183 317 1968

12 Lewis E J Blaufox M D Jagger P Lauler D Qureshi E & Merrill J P Sodium balance in the human renal transplant J Clin Invest 45 1040 1966

13 Liard J F & Peters G Mechanism of the fall in blood pressure after unclamping in rats with Gold blatt type of hypertension Experientia (Basel) 26 743 1970

14 Lundgreen C Bozovic L & Castenfors J Plasma renin activity before and after allogenic kidney trans plantation in man Scand J Urol Nephrol 1 181 1967

15 Margules R M Belzer F O & Kountz S L Surgical correction of renovascular hypertension fol lowing renal allotransplantation Arch Surg 106 13

16 Morns P J Yadav R V S Kincaid Smith P Anderton J Hare W S C Johnson N Johnson W & Marshall V C Renal artery stenosis in renal transplantation Med J Aust 1 1255 1971
17 Newton W T Keltner R M & Schankel S W Acquired renovascular hypertension in a patient with *renal allotransplantation* Am J Surg 113 292 1967
18 Nielsen I Clausen E & Jensen G Plasma renin activity after allogenic kidney transplantation in man Acta Med Scand 188 355 1970
19 Papadimitriou M Chrisholm G D & Shackman R Hypertension in patients on regular haemodialysis and after renal allotransplantation Lancet 2 902 1969
20 Popovtzer M M Pinnggera W Katz F H Corman J L Robinette J Lanois B Halgrimson C G & Starzl T E Variations in arterial blood pressure after kidney transplantation Relation to renal function plasma renin activity and the dose of prednisone Circulation 47 1297 1973
21 Rask Madsen J Bruusgaard A Munck O Nielsen M D & Worning H The significance of bile acids and aldosterone for the electrical hyperpolarization *of human rectum in obese patients treated* with intestinal bypass operation Scand J Gastroenterol 9 417 1974
22 Roguska J DelGreco F & Simon N M Serum renin activity in human renal homotransplantation Nephron 8 289 1971
23 Sampson D Kirdani R Y Sandberg A A Murphy G P The aetiology of hypertension a renal transplantation in man Br J Surg 60 819 19
24 Smellie W A B Vinik M & Hume D M Ang graphic investigation of hypertension complica human renal transplantation Surg Gynecol Ob 128 963 1969
25 Staple T W & Chiang D T C Arteriography f lowing renal transplantation Am J Roentgenol 1 669 1967
26 Tobian L Coffee K & McCrea P Contrast exchangeable sodium in rats with different types Goldblatt hypertension Am J Physiol 217 458 19
27 Verniory A Potvliege P Van Geertruyden J Vereerstraeten P Kinnaert P Staroukine M Toussaint C Renin and control of arterial blo pressure during terminal renal failure treated haemodialysis and by transplantation Clin Sci 685 1972
28 Wallenius M Fyhrquist F & Kock, B Plas renin activity in the evaluation of hypertension renal transplant recipients Scand J Urol Neph (Suppl) 147 1975
29 *West T H Turcotte J G & Vander A J* Plas renin activity sodium balance and hypertension i group of renal transplant recipients J Lab Clin M 73 564 1969

Acta Med Scand 202 517–521 1977

Hemodynamic Effect of Long-term Treatment with Pindolol in Essential Hypertension with Special Reference to the Resistance and Capacitance Vessels of the Forearm

Jan Henrik Atterhog Hans Duner and Bengt Pernow

From the Department of Clinical Physiology Karolinska Hospital and the Department of Medicine Sabbatsberg Hospital Stockholm Sweden

ABSTRACT Ten patients with essential hypertension have been studied at rest, during and after exercise following oral treatment for on an average 16 months with a β-adrenoreceptor blocking agent pindolol The study was a direct continuation of an earlier, performed after 2 months' treatment The hypotensive effect of pindolol was highly significant after 2 as well as 16 months of treatment Heart rate was similarly lowered at 2 and 16 months, while cardiac output, which was significantly lower during exercise after 2 months had increased to the pretreatment level after 16 months Peripheral vascular resistance, which was not affected after 2 months had decreased significantly during and after exercise in the long term study A comparison between the hemodynamic situations after 2 and 16 months thus suggests that while a decrease in cardiac output is an early mechanism in the lowering of BP, changes in systemic vascular resistance seem to be more important after long term treatment with pindolol

Pindolol (Visken® Sandoz) is a potent β adrenoreceptor blocking agent (14) with a weak quinidine like effect (15) a receptor stimulating or intrinsic activity (4) no direct effect on α adrenoreceptors (4) and a long duration of the effect measured as the inhibition of isoprenaline induced tachycardia (18)

The efficiency of pindolol in lowering the BP in hypertension has been documented in a number of reports (5 17 21 22) The above mentioned long duration of the effect also seems to apply to the hypotensive effect since administration of pindolol twice (25) and even once a day (11) has been used in the treatment of hypertension with satisfactory results

It is generally agreed that the major mechanism behind the short term hypotensive effect of β blockers is a reduction of cardiac output as a consequence of induced bradycardia (1 10 16 24) The effect of β blockers on the peripheral resistance seems on the other hand to vary somewhat depending on which preparation has been used Thus systemic vascular resistance is reported to be initially increased by propranolol (10 24) while this increase is less pronounced after alprenolol (12) practolol (13) or metoprolol (19) After short term treatment of essential hypertension with β adrenoreceptor blockers systemic vascular resistance is reported to decrease towards the pretreatment value on propranolol (10 24) or below the control value on e g alprenolol (20) and pindolol (1)

In a long term follow up with propranolol (24) systemic vascular resistance decreased continuously with time from the initial peak though it did not fall significantly below the pretreatment level

The aim of the present study was to investigate the hemodynamic effect of long term treatment with pindolol—mean 16 months—in essential hypertension Besides arterial BP cardiac output and systemic vascular resistance the effects on blood flow vascular resistance and venous tone of a peripheral area (forearm) were also studied The study is a direct continuation of a previous investigation on the same patients after 2 months of treatment with pindolol (1)

STUDY BASE

The study comprised ten patients (5 women and 5 men) with essential hypertension (stages I and II according to

Table I *Central hemodynamics and peripheral circulatory data at rest during and 4 min after exercise before (A) and after 2 months (B) and 16 months (C) on pindolol*

	Rest				Exercise				4 min after exercise			
	A	B–A	C–A	C–B	A	B–A	C–A	C–B	A	B–A	C–A	C–B
Arterial BP (mmHg)												
Systolic												
Mean	193	−34	−40	−6	229	−47	−54	−7	179	−29	−31	[illegible]
S D	22				21				23			
p		<0 001	<0 001	n s		<0 001	<0 001	n s		<0 001	<0 001	n s
Diastolic												
Mean	106	−19	−21	−2	117	−24	−29	−5	105	−21	−20	1
S D	14				11				12			
p		<0 001	<0 001	n s		<0 001	<0 001	n s		<0 001	<0 001	n s
Mean												
Mean	140	−23	−27	−4	159	−29	−33	−4	134	−21	−23	[illegible]
S D	13				14				14			
p		<0 001	<0 001	n s		<0 001	<0 001	n s		<0 001	<0 001	n s
Heart rate (beats/min)												
Mean	74	−8	−7	1	119	−18	−18	0	84	−9	−7	[illegible]
S D	12				20				14			
p		<0 01	<0 05	n s		<0 01	<0 01	n s		<0 05	<0 05	n s
Cardiac output (ml/min)												
Mean	5 5	−0 7	−0 4	0 3	10 4	−1 4	−0 4	1 0	6 1	−0 3	±0	0 3
S D	1 3				2 5				1 2			
p		<0 05	n s	n s		<0 001	n s	<0 05		n s	n s	n s
Stroke volume (ml)												
Mean	75	−1	1	2	89	±0	11	11	76	2	3	±0
S D	17				21				16			
p		n s	n s	n s		n s	<0 05	<0 05		n s	n s	n s
Systemic vascular resistance (arb u)												
Mean	27	−2	−3	−1	16	−1	−3	−2	23	−3	−4	−1
S D	8				5				5			
p		n s	<0 05	n s		n s	<0 001	<0 01		<0 05	<0 001	n s
flow (ml×100⁻¹ ml×min⁻¹)												
Mean	2 8	0 1	0 4	0 3	2 8	−0 2	0 9	1 1	3 6	±0 0	0 6	0 6
S D	1 2				1 2				1 7			
p		n s	n s	n s		n s	n s	<0 05		n s	n s	n s
Forearm vascular resistance (arb u)												
Mean	60	−14	−24	−10	65	−14	−30	−16	45	−10	−16	−6
S D	28				27				21			
p		n s	<0 05	n s		<0 05	<0 05	<0 05		n s	<0 05	n s
Venous tone (mmHg×ml⁻¹×100 ml)												
Mean	3 0	−1 0	−0 7	0 3	2 9	−0 8	−1 1	−0 3	3 3	−1 5	−1 8	−0 3
S D	2 0				1 7				2 3			
p		<0 05	n s	n s		<0 01	<0 05	n s		<0 05	<0 05	n s

the WHO) Their mean age was 47 8 years (range 31–61) when entering the first study (1) and 49 2 years at the time of the present investigation carried out 52–73 weeks (mean 16 months) after a placebo period The daily dosage of pindolol—divided in two—was 20 mg in one patient 30 mg in four patients and 40 mg in five The individual dosages of pindolol were the same in the two studies No other antihypertensive drugs were given Further details concerning the patients are given in the previous study (1)

METHODS AND PROCEDURE

The studies were performed in the morning with the patient in the supine position at rest during exercise on a bicycle ergometer and 4 min after work The patients had taken their pindolol dosage 2 hours before the examination but were otherwise fasting The examination was carried out in exactly the same manner as described earlier (1), using the same work load on the bicycle The arterial BP

Table II *Plasma renin activity (ng×100 ml⁻¹×h⁻¹) before and after 2 months and 16 months of pindolol treatment (mean±S D)*

	Control value	Pindolol treatment		*P*	
		2 mo	16 mo	16 mo –control	16 mo –2 mo
Early in the morning in the ward	0 98±0 82	0 26±0 28	0 43±0 51	n s	n s
In connection with the hemodynamic study					
Before exercise	1 85±1 02	0 55±0 33	0 36±0 37	<0 001	n s
1 min after exercise	1 96±1 49	0 80±0 54	0 32±0 33	<0 001	<0 05

was recorded via a catheter inserted in the right brachial artery Heart rate was measured from the ECG recorded from a precordial lead Cardiac output was estimated by the dye dilution technique Forearm venous pressure was measured through a catheter placed in a deep vein in the left forearm and forearm blood flow by venous occlusion plethysmography Systemic vascular resistance was calculated as the ratio of mean brachial artery pressure to cardiac output and expressed in arbitrary units Forearm vascular resistance was calculated as the ratio of mean brachial BP to forearm blood flow Venous tone of the forearm was defined as the ratio of increase in pressure ($\Delta P/\Delta T$) to increase in volume ($\Delta V/\Delta T$) and expressed as mmHg×ml⁻¹×100 ml tissue

Samples for determination of plasma renin activity (PRA i e angiotensin I) were taken in the morning with the patient resting in bed and also in connection with the hemodynamic study immediately before and after exercise

The results are presented as mean values ± S D Statistical significance of differences is evaluated by *t* tests on paired observations

RESULTS

All ten patients tolerated pindolol well without side-effects as reported in the first study (1) Serum creatinine ASAT ALAT electrolytes WBC RBC and trombocytes were unaffected by the pindolol treatment In the two patients in whom a slight increase in heart volume was noted before and after 2 months of treatment the condition was unchanged in the 16-month study

Comparison between placebo treatment and 16 months of pindolol treatment

The arterial systolic diastolic and mean BPs and heart rate were all significantly lower at rest and during exercise after 16 months of pindolol treatment compared with the control situation (Table I) There was a significant increase in stroke volume during exercise whereas cardiac output was the same as in the control study both at rest and during exercise

Both systemic vascular resistance and vascular resistance of the forearm were significantly lower after 16 months on pindolol than before at rest during and after exercise (Table I) Forearm blood flow was not significantly changed (Table I) Venous tone was significantly decreased during and after exercise

PRA was significantly decreased during pindolol treatment immediately before and 1 min after exercise (Table II)

Comparison between 2 months and 16 months of pindolol treatment

The systolic diastolic and mean arterial BPs were numerically lower after 16 months than after 2 months but the difference was in no instance significant (Table I) Heart rate was unchanged at rest during and after exercise Cardiac output and stroke volume were somewhat higher than after 2 months though the difference was significant only during exercise

Systemic as well as forearm vascular resistance were lower after 16 months on pindolol treatment than after 2 months the difference being significant during exercise (Table I) No difference was found regarding venous tone

The changes in forearm blood flow between the examinations after 2 and 16 months during and after exercise co-varied with the corresponding changes in cardiac output The *r* value during exercise was 0 71 ($p<0\ 05$) and after exercise 0 66 ($p<0\ 10$) PRA was significantly lower after exercise (Table II)

In a 50-year old woman whose BP had been unchanged at the two-month examination a decrease to a normal pressure level was recorded after 16

months on pindolol whereas both heart rate and cardiac output were unchanged compared with the 2 month values

DISCUSSION

It is evident from our previous report (1) and the present results that in the treatment of essential hypertension with pindolol at least three mechanisms seem to be involved in the decrease in BP These are 1) a negative chronotropic effect on the heart 2) a reduced peripheral vascular resistance and 3) a decrease in venous tone

During the early stage of treatment the most important factor behind the reduction of BP was evidently the decrease in cardiac output This decrease was a consequence of the negative chronotropic effect of pindolol while stroke volume was unaffected probably partly due to the simultaneous decrease in venous tone After 16 months however the cardiac output had returned to a level not significantly different from that during the placebo medication But the calculated vascular resistance showed a significant decrease which seems to indicate that the lowered BP level after long term treatment mainly depends upon a decreased peripheral vascular resistance

The decrease in peripheral resistance after olol treatment may be hypothetically explained various mechanisms It may be an effect on tral nervous mechanisms regulating the tone of smooth muscles in the resistance vessels A direct effect through the intrinsic activity of pindolol on the β receptors in these vessels may also be of significance But in view of the successive reduction of the peripheral resistance it is tempting to interpret this as a reversibility of structural changes in the resistance vessels Evidence has been accumulated concerning the existence of such structural changes in hypertension and also about their reversibility Experience from the spontaneous hypertensive rat (26) has shown that the elevated BP induces structural changes in the precapillary resistance vessels thereby increasing the peripheral resistance In such rats (7) as well as in humans with essential hypertension (6 23) a higher vascular resistance is still present at maximal vasodilatation i e when all vasoconstrictor nerve activity and myogen auto tone are abolished The reversibility of these structural changes has been debated Weiss (26) observed that in spontaneously hypertensive rats β blockers are able not only to prevent the development of structural changes but also to reduce to some extent already established alterations A corresponding reversibility of the vascular resistance in hand circulation has been shown recently in hypertensive patients after hypotensive treatment (9) It is thus possible that the decrease in peripheral resistance during pindolol treatment in our patients might indicate a partial regress of structural changes in the resistance vessels

The effect on forearm blood flow exerted by supine leg exercise is normally an unchanged or decreased flow, depending on the work load due to an α adrenergically mediated increase in forearm vascular resistance (2 3) The patients in our studies reacted normally in this respect indicating an increase in α adrenergic tone during exercise However despite an unchanged level of perfusion pressure during treatment with pindolol there was a significant increase in forearm blood flow during exercise after 16 months of treatment but a significant decrease in forearm vascular resistance Compared with the placebo study forearm vascular resistance was significantly lower also at rest and after exercise These results might also conform with a regress of structural changes in the resistance vessels even though a decreased α adrenergic tone after 16 months on pindolol cannot be excluded

There are reports of patients with essential hypertension who are resistant to a β blocker regimen (8 24) In our series a successful regress of hypertension was demonstrated in a 59 year-old woman after 16 months on an unchanged dose of pindolol although the BP level was unchanged after 2 months of therapy Decreased PRA levels indicated that this patient had actually taken her medicine at the first hemodynamic control This case illustrates that the hypotensive effect may occur after a long period on an unchanged regimen

As mentioned in the introduction most reports concerning the mechanism of the BP lowering action of β blockers have given prominence to the significance of a decrease in cardiac output Only exceptionally has a decrease been reported in the peripheral vascular resistance to values below the pretreatment level However it seems essential to emphasize that the β blockers are a group of substances which differ in many of their properties These differences and the alteration of the hemodynamic situation during long term treatment

complicate comparisons between different β blockers and different studies

REFERENCES

1 Atterhog J H Dunér H & Pernow B Hemodynamic effect of pindolol in essential hypertension with special reference to the resistance and capacitance vessels of the forearm Acta Med Scand 199 251 1976
2 Bevegård S & Shepherd J T Reaction in man of resistance and capacity vessels in forearm and hand to leg exercise J Appl Physiol 21 123 1966
3 Blair D A Glover W E & Roddie J C Vasomotor responses in the human arm during leg exercise Circ Res 9 264 1961
4 Clark B & Saameli K Ein neuer β Receptoren Blocker Triangle 9 300 1970
5 Dunér H The effect of LB 46 on the blood pressure in essential hypertension Indian Heart J (Suppl) 1 236 1972
6 Folkow B Grimby G & Thulesius O Adaptive structural changes of the vascular walls in hypertension and their relation to the control of the peripheral resistance Acta Physiol Scand 44 255 1958
7 Folkow B Hallback M Lundgren Y & Weiss L Structurally based increase of flow resistance in spontaneously hypertensive rats Acta Physiol Scand 79 373 1970
8 Frolich E Tarazi R Dustan H & Page I The paradox of beta adrenergic blockade in hypertension Circulation 37 417 1968
9 Hansson L & Sivertsson R Reversibility of structural vascular changes in human essential hypertension In Pathophysiology and management of arterial hypertension (ed G Berglund L Hansson & L Werko) p 14 Lindgren & Soner Mölndal Sweden 1975
10 Hansson L Zweifler A J Julius S & Hunyor S N Hemodynamic effects of acute and prolonged β adrenergic blockade in essential hypertension Acta Med Scand 196 27 1974
11 Jacobsson K A Antihypertensiv terapi med pindolol – en jamförelse mellan två doser och en dos per dag Lakartidningen 73 2500 1976
12 Johnsson G de Guzman M Bergman H & Sannerstedt R The haemodynamic effects of alprenolol and propranolol at rest and during exercise in hypertensive patients Pharmacol Clin 2 34 1969
13 Leon D Thompson M Shaver J & McDonald R Hemodynamic effects of practolol at rest and during exercise Circulation 45 46 1972
14 Levy J Cardiovascular effects of prindolol (LB-46) a potent beta adrenergic receptor antagonist J Clin Pharmacol 11 249 1971
15 Lubawski I & Wale J Studies with PB 46 a new β receptor blocking drug Eur J Pharmacol 6 345 1969
16 Lund Johansen P Hemodynamic changes at rest and during exercise in long term β blocker therapy of essential hypertension Acta Med Scand 195 117 1974
17 Morgan T O Louis W J Dawborn J K & Doyle A E The use of pindolol (Visken) in the treatment of hypertension Med J Aust 59 309 1972
18 Olsson S B & Varnauskas E Duration of beta receptor blockade after oral administration of LB-46 Eur J Clin Pharmacol 5 214 1973
19 Sannerstedt R Haemodynamic effects of adrenergic β-receptor blocking agents in arterial hypertension In Pathophysiology and management of arterial hypertension (ed G Berglund L Hansson & L Werko) p 194 Lindgren & Soner Molndal Sweden 1975
20 Sannerstedt R Stenberg J Vedin A Wilhelmsson C & Werko L Chronic beta adrenergic blockade in arterial hypertension Hemodynamic influences of dihydralazine and dynamic exercise and clinical effects of combined treatment Am J Cardiol 29 718 1972
21 Seedat Y K & Stewart Wynne E Clinical experiences with pindolol (Visken) in the therapy of hypertension S Afr Med J 46 1524 1972
22 Simpson S O & Waal Manning H J Hypertension and beta adrenergic blockade In New horizons in medicine (1) Symposium Beta Adrenergic Blocking Agents p 59 Sandoz Sydney 1970
23 Sivertsson R The hemodynamic importance of structural vascular changes in essential hypertension Acta Physiol Scand (Suppl) 343 1970
24 Tarazi R & Dustan H Beta adrenergic blockade in hypertension Practical and theoretical implications of long term hemodynamic variations Am J Cardiol 29 633 1972
25 Waal Manning H J & Wood A J Pindolol in hypertension Twice-daily versus thrice-daily dosage Med J Aust 2 274 1975
26 Weiss L Aspects of the relation between functional and structural cardiovascular factors in primary hypertension Experimental studies in spontaneously hypertensive rats Acta Physiol Scand (Suppl) 409 1974

ANNOUNCEMENTS

The International Prize for Modern Nutrition amounting to 15 000 – Sfr will be awarded in Sept 1978 and Sept 1979 by the Central Union of Swiss Milk Producers Berne for recent research work on the following subjects *1978* Importance and value of milk and milk products in the nutrition of the elderly Filling date of application Feb 15 1978 *1979* Social and psychological aspects of the selection of nutrition Filling date of application Feb 15 1979 By publishing the subject for 1979 in advance the donor aims to stimulate new research work which corresponds to this goal of health education

All scientists from the following countries who have worked in these fields are eligible for the prizes Argentina Australia Austria Belgium Brazil Bulgaria Canada Czechoslovakia Denmark Finland France India Ireland Israel Italy Japan Kenya Luxemburg Netherlands New Zealand Norway Poland South Africa Spain Sweden Switzerland United Kingdom USSR West-Germany

Applications should be sent to the President of the jury Professor M Demole 4 chemin Castoldi CH 1208 Genève Switzerland with 3 copies of curriculum vitae list of works reprints of 2 or 3 papers on the subject of the prize published in the last 5 years (no typewritten papers) These documents should be written in English French or German or should be accompanied by a translation into one of these 3 languages

International Symposium on Genetic Engineering Scientific Developments and Practical Applications will be held in Milan Italy March 29–31 1978 organized by Fondazione Giovanni Lorenzini Milan Italy and the WHO Geneva Switzerland The official language will be English

Preliminary programme Opening session Developments and trends in genetic engineering Basic technological aspects (modification and improvement of microorganisms in general modification and improvement of industrial microorganisms preparation of suitable vectors preparation of suitable hosts modern approaches to gene control) Practical applications (immunology and pharmacology analysis of genetic structure agriculture the view of industry special applications for developing countries) International cooperation (diffusion of information safety measures codes of practice training and containment) Major conclusions and recommendations Adoption of the report Closing session

Secretariat Fondazione Giovanni Lorenzini Via Monte Napoleone 23 I 20121 Milan Italy

Acta Med Scand 202 523–528 1977

Management of Septicemia and Early Death in Acute Leukemia

Bo Lantz and Peter Reizenstein
with technical assistance of Kerstin Jakobsson

*From the Division of Hematology Department of Medicine
Karolinska Hospital Stockholm Sweden*

ABSTRACT The frequency of fever days was measured in 67 patients with acute leukemia in the initial phase until remission was obtained or the patient died A total of 3411 hospital days were studied Three antibiotic schedules were examined between 1970 and 1975 There were 50% fever days when penicillin and streptomycin were combined as initial antibiotics 37% when cephalosporin and gentamycin were used and 31% when, in addition semi isolation was used prophylactically and granulocytes were given in a therapeutic attempt (0 05>p>0 01) The corresponding frequencies of early death were 9/21 (43%), 6/23 (26%) and 5/23 (22%) Early death occurred on an average 14, 23 and 21 days after admission The frequencies of remission (complete and partial) were 8/21 (38%), 12/23 (52%), and 13/23 (57%) A randomized subgroup (12 patients) with intestinal sterilization was studied separately It had 28% fever days, which is significantly less (p<0 01) than in the 13 patients given the same systemic antibiotics without intestinal sterilization Fever was significantly lower (0 05>p>0 01) on the day after a series of *granulocyte transfusions* than before although only 10^9 granulocytes were given However, this fever reduction may have been due to concomitant antibiotics

To be able to administer adequate doses of cytostatic agents over adequate periods in acute leukemia one must prevent early death from complications (27) Well described complications are septicemia (2 3 7 9 11 15 18 19 25 26) electrolyte disturbances (16 23 30) hyperuricemia (21) bleeding because of thrombocytopenia or coagulopathy (1 22 28) It has been demonstrated that thrombocytopenia can be managed for prolonged periods *with the aid of thrombocyte transfusions (13 17)* The clinical relevance and role of hypokalemia are still being studied The present retrospective study will describe a local attempt to prevent and treat septicemia and early death

PATIENTS

Sixty seven patients with previously untreated acute leukemia are presented in Table 1 All were treated in the same department of medicine between 1970 and 1975 Only the initial phase of the disease ending with remission or death is described here Later relapses are included for only two purposes when the immediate effect of granulocyte transfusions on fever is discussed and when the early deaths are compared with the later ones

METHODS

Five methods were tested for preventing or treating septicemia

1 Penicillin–streptomycin Bacteriological specimens from nose throat sputum urine and possible known infectious foci were obtained immediate treatment was started with penicillin and streptomycin and we changed to relevant antibiotics as soon as a plausible causative microorganism and its sensitivity had been established

2 Cephalosporin–gentamycin The antibiotics above were replaced by antibiotics effective against gram negative enterobacilli Initially streptomycin was used in combination with cephalosporin (Keflin® Lilly) If this was ineffective gentamycin (Garamycin® Schering) was used and if this failed ampicillin (Doktacillin® Astra) in combination with carbenicillin (Fugacillin® Astra)

3 Semi isolation and granulocytes Prevention of septicemia was attempted by isolating patients as soon as the granulocyte count was under 150/mm³ Isolation consisted of a single room equipped with an ante room in which shoes were decontaminated with National Cash Register antidust mats whose absorptive layer was

Table I *Patient stratification intestinal sterilization*

	Treatment schedule				
	Peni cillin–streptomycin	Cephalo-sporin–gentamycin	Semi isola tion and granulocytes	Total	Intestinal sterilization
No of pats	21	23	23	67	12
Males	9	10	10	29	6
Females	12	13	13	38	6
>70 y	6	4	3	13	4
>80 y	0	1	2	3	
Myeloblastic	15	15	11	41	8
Promyelocytic	0	2	6	8	1
Monomyelocytic	1	0	2	3	0
Lymphoblastic	4	3	1	8	2
Unclassified	1	0	0	1	0
Erythroleukemic	0	3	3	6	1

changed every 4th day where hands were washed and desinfected gowns changed and masks donned However no separate ventilation laminar air flow or life islands were used nor were foods drugs or other materials entering the room sterilized Each patient had separate toilet and washing facilities

In combination with the isolation procedure described above simplified granulocyte transfusions were given to patients with less than 150 granulocytes/mm³ and fever who were receiving antibiotics The simplified granulocyte transfusions consisted of the buffy coat from 6 ...ss match compatible units of blood and contained 10^9 ... approximately Genuine granulocyte transfusions ...taining approximately 10^{11} granulocytes isolated with a separator or a filter were not available

4 Intestinal sterilization A randomized sample (12 patients) of the 23 patients given cephalosporin–gentamycin also received systemic antibiotic treatment with intestinal sterilization with a combination of neomycin bacitracin and nystatin Sterilization was controlled in fecal cultures

5 Antibiotic prophylaxis During 1974–75 trimethoprimsulpha and phlucytosine were given to 12 and 6 ...tients respectively Normal oral dosages were given fo... least one month

Study of different treatments

The testing was performed in sequence (Table ... Twenty one patients (group I) were treated with peni...lin–streptomycin during 1970–71 During 1972–73 23 ...tients (group II) were treated according to method 2 (...phalosporin–gentamycin) During 1973–75 23 patie... (group III) were likewise treated with cephalosporin–g...tamycin and in addition with semi isolation and granu...cytes

Whereas during earlier periods patients had been ...tributed on different wards in the Department ... Medicine a Hematology Division was established in 19... where the staff was stable and trained

Overlapping and restrictions A period of change had ... be allowed for between the treatment schedules granu...cyte transfusions for instance were tested in 2 patie... during 1972–73 before being made a *standard treatme...*

Table II *Cytostatics used*

Penicillin–streptomycin Group I 1970–71	Cephalosporin–gentamycin Group II 1972–73 (June)	Semi isolation and granulocytes Group III 1973 (July)–75
Cytosine arabinoside (2 mg/kg/day×4 days)	Cytosine arabinoside (2 mg/kg/day×5 days)	Rubidomycin (1 5 mg/kg/day×1 day)
Cyclophosphamide (100 mg/m²×4 days)	Cyclophosphamide (100 mg/m²×4 days)	Cytosine arabinoside (2 mg/kg/day×5 days)
Precortalone (2 mg/kg/day×4 days)	Precortalone (2 mg/kg/day×5 days)	Precortalone (3 pt) (2 mg/kg/day×5 days)
Rubidomycin (1 pt) (1 5 mg/kg/day×1 day)	Rubidomycin (1 5 mg/kg/day×1 day)	Thioguanine (1 pt) (2 mg/kg/day×5 days)
	Asparaginase	Asparaginase (2 pt)

Table III *Frequency of fever days and results of treatment*

Treatment	No of pats	Per cent of total days with fever			Deaths within 6 week	Remissions	
		38–39°C	39–40°C	>40°C		Complete	Partial
Penicillin–streptomycin	21	30	15	5	9	5	3
Cephalosporin–gentamycin	23	24	10	3		11	1
Isolation–granulocytes	23	20	8	3	5	11	2

some overlapping in therapy is described in Table II One patient in group I did receive rubidomycin Three patients in group I did not receive antibiotics at all Intestinal sterilization was given to two patients belonging to group These two patients had an early death

Concomitant therapy In addition to the anti infectious measures just described all patients received required supportive treatment in the form of erythrocyte and platelet transfusions Cytostatic therapy is described in Table II It consisted of cytosine arabinoside prednisolone and cyclophosphamide in 1970–71 In 1972 rubidomycin–cytosine arabinoside was introduced Patients over years of age were given thioguanine in combination with cytosine arabinoside If the treatment tested initially failed the next trial in all periods consisted of vincristine prednisolone 6-merkaptopurine and methotrexate Later cyclophosphamide vincristine methotrexate and prednisolone combination or one of cyclophosphamide merkaptopurine and thioguanine was used To obtain remission 1–5 courses were required in 1970–71 3–5 in 72–73 and 3–9 in 1973–75

and definitions

All data were obtained from the clinical and autopsy reports In 7 cases autopsy was not allowed by the relatives Three patients were living in another county Four patients are still alive

Fever is defined here as a temperature of more than C The highest temperature on each day was noted The number of fever days is related to the total number of days in hospital from admission to remission or death In a separate part of this study the effect on fever of granulocyte transfusion was studied in a total of 26 patients and 195 transfusions A total of 82 series were given in connection with fever The highest fever on the day before and after the transfusion series was noted Each series contained an average of [illegible] (range 1–10) transfusions One transfusion each day Not all [illegible] transfusion given in the initial phase of the disease this is on two only parts of the study where relapse phase leukemia after an initial remission [illegible] included

Early death is defined as death within 6 weeks of admission *Marrow aplasia* refers here only to [illegible] findings *Cause of death* means the autopsy diagnoses [illegible] diagnoses The early deaths are compared with the ones and this is the other part of the present study [illegible] the relapse phase is included Severe *bicytopenia* [illegible] fined as a WBC below 1 500 and a thrombocyte count below 15 000/mm³

RESULTS

Effect of penicillin–streptomycin as initial antibiotics The leukemia patients had fever over 38 C on 50% of hospitalized days during the period when method 1 was used on 20% of the days over 39°C Forty three per cent of the patients died during the first 6 weeks (early death) and 24% achieved a complete remission (Table III)

Effect of cephalosporin–gentamycin During the time when the antibiotics directed against gram negative bacilli were used the frequency of days with fever over 38°C was 37% and over 39°C 13% The frequency of early death was reduced to 26% and the number of complete remissions increased to 48% The difference between groups 1 and 2 is not statistically significant

Effect of intestinal sterilization Intestinal sterilization was given to 10 of the 23 patients treated with cephalosporin–gentamycin and to 2 early death patients in group 3 (621 hospital days) The intestinal sterilization subgroup had only 28% fever days and fever over 39°C occurred in 10% of the hospital days Compared with the 13 non sterilized patients (627 hospital days) in whom corresponding percentage was 28 against 18 there is a significant difference in fever days ($p<0.01$) for fever up to 39°C

Table IV *Causes of early and late death*

	Hemorrhage		Septicemia	Combined	Other
	Cerebral	General			
Early death (n=20)	5	1	6	4	4
Late death (n=3?)	4	6	12	5	6

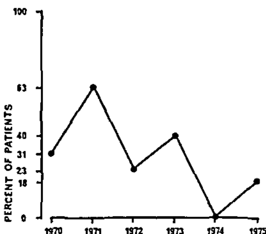


Fig 1 Early death by years

Effect of isolation and granulocyte transfusion During the period when these methods were used the frequencies of fever days with fever over 38 C and over 39°C were 31% and 11% respectively of the days in hospital The early death rate was further decreased to 22% The complete remission rate remained the same as in the undivided group II but the combined remission rate was 57% Com ... with group I the decrease in fever over 39°C ... tistically significant ($p<0.05$)

... ocyte transfusion and fever The mean ... emperature before a series of granulocyte ... usions was 38 9°C and on the day after the ... 38 5 C The difference was statistically ... ificant ($p<0.05$)

Early death Even the patients who died within 6 weeks died in a short time during the penicillin–streptomycin period (after 14 days) as compared with those who died during later regimes after averages of 21 and 23 days

The cause of early death seems to differ from that in the patients living longer (Table IV) A comparison between the 20 early and 33 late cases of death in which autopsy was performed shows that fatal cerebral bleedings caused 5 (25%) of the early deaths compared with only 12% of the late deaths In contrast fatal general bleeding caused 18% of the late but only 5% of the early deaths Two of the 4 patients who died late due to fatal cerebral hemorrhagia lived slightly longer than those who died early (after 44 and 58 days respectively) Aplasia and bicytopenia were equally distributed among early and late deaths

DISCUSSION

This is a retrospective study using historical c... trols and it must be interpreted as such A prosp... tive randomized study would have been simple... interpret but would have made it difficult to fol... the rapid development in treating acute leuker... For instance it would clinically have been alm... impossible to persist for statistical reasons in ... use of penicillin as a first antibiotic after 1971 o... justify the non use of rubidomycin after 1972 (6...

It has been stated that the change between ... *treatment schedules* was sometimes *gradual* ... that the establishment of a Hematology Divisi... made it possible to train the staff For instance ... semi isolation routine was probably broken mo... often initially than later when everyone had be... trained to adhere to it

Every effort will be made in the discussion bel... to consider the possible effect of these factors ... the results of treatment The patient stratificati... (Table I) suggests acceptable comparability b... tween the different treatment periods as regar... number of patients age, sex and cell morpholog... The penicillin–streptomycin group includes ... erythroleukemias—a prognostically perhaps u... favorable group—but more lymphoblastic le... kemias—a prognostically probably favorable gro... —*than the later treatment periods*

In the division studied here frequency of fe... decreased steadily from 1970 to 1975 Likew... there was a continual decrease in the number ... early deaths (Table III Fig 1) It has already be... stated that cytostatic treatment varied to some ... tent during this period as did the general manag... ment of the patients However we feel that none ... these factors are a more likely explanation of t... improved results than the change in antibiotics ... commended also by others in recent years (5 1... 20) On the other hand the present study does n... permit the conclusion that the semi isolation us... reduces infections or increases the remission ra... significantly

Only the frequency of fever days was reduce... *not the absolute number* since the mean hospi... stay increased during 1970–75 It is suggested th... the frequency reflects therapeutic success bett... than the number since its development parallels th... reduction of the number of early deaths and th... increase in the number of remissions

Granulocyte transfusion Granulocyte trans... sions containing only 10^9 cells could be demo...

ed to reduce the fever temporarily However it n whether the reduct on of fever is due to tes or to concomitant antibiotic treat

Pre ous stud es on the effect of granulocyte ons have given contradictory results gby et al (14) found a more marked reduction of when granulocytes and antibiot cs were given when only antibiotics were used and they this effect even without an increase in the ent s WBC Neither of these f ndings could be rmed however (4 10 24) The authors quoted 10 ° 10 ¹ granulocytes which is 10–100 more than our dose The efficacy of trans with 10⁹ granulocytes is therefore st ll un

est nal sterilizat on The subgroup with in nal sterilizat on had a significantly lower fre ncy of fever days This group was randomized the result is therefore more rel able than in our r groups The frequency of rem ss on was not if cantly better wh ch is in accordance with the lts of others (29)

ACKNOWLEDGEMENT

by the Swed sh Cancer Soc ety (grant no 76

REFERENCES

Amem ya Y Abnormal t es of blood coagulat on and fibrinolys s n acute leukemia—their influence on bleed ng tendency Acta Haematol 38 143 1975

Armstrong D Young L S Meyer R D & Blevine A A Infect ous compl cations of neoplast c d sease Med Cl n North Am 55 729 1971

Atk nson K Kay H E M & McElwa n T J Sept cem a in the neutropenic pat ents Br Med J 3 244 1974

Bodey G P Buckley M Sathe Y S & Fre re ch E J Quantitat ve relat onsh ps between c rculat ng leukocytes and infect on in pat ents w th acute leukem a A n Intern Med 64 328 1966

Britt nger G Unspezif sche Bas stherap e der Leu kämien und ihrer Kompl kat onen Med Kl n 69 34 1344 1974

Buchanan J Matthews J Dawison J Grigor R & Casey T Treatment of acute myelo d leukem a of adults with cytos ne arabinos de in comb nat on w th 6-merkaptopurine Cancer 32 789 1973

Chi C H Lagerlöf B Lantz B & Re zenste n P Autopsy f nd ngs n leukaem a Scand J Haematol 17 251 1976

Cooperat ve Leukemia and Hematosarcoma Group of the EORTC (Hop tal Paul Brousse Villej f France) Comparison of the effect of cytos ne arab nos de of cytos ne arab nos de and th oguan ne and of cytos ne arab nos de and daunorub c ne n acute myelo d leukem a (in French) Bull Cancer (Paris) 61 411 1974

9 Flem ng P C Bacterial infection in leukemia Cancer in ch ldhood p 2?9 Plenum Press New York 1973

10 Fortuny I E Bloomf eld C D Hadlock D C Goldman A Kennedy B J & McCullough J J Granulocyte transfus on A controlled study in pa t ents w th acute nonlymphocyt c leukem a Transfu s on 15 548 1975

11 Frei E Lev n R H Bodey G P Morse E E & Fre reich E J The nature and control of infect ons in pat ents w th acute leukem a Cancer Res 25 1511 1965

12 Freire ch E J Bodey G P McCred e K B Rodriguez V Hart J S Burgess M A & Gut terman J U Management of acute leukem a Proc Natl Cancer Conf 7 345 1973

13 H gby D J Cohen E Holland J F & S nks L The prophylact c treatment of thrombocytopen c leukem c pat ents w th platelets a double bl nd study Transfus on 14 440 1974

14 H gby D J Yates J W Henderson E S & Hol land J F Filtrat on leukopheres s for granulocyte transfus on therapy N Engl J Med ?9? 761 1975

15 Hersh E M Bodey G P N es B A & Fre re ch E J Causes of death in acute leukem a JAMA 193 105 1965

16 Höcker P & Re zenste n P Cal ium and potass um d sturbances in acute leukem a. Blut ?9 398 1974

17 — Effect on platelet counts and fever of platele transfus ons in acute leukem a Blut 31 143 1975

18 Hughes W Fatal infect ons in ch ldhood leukem a Am J D s Child 12? 283 1971

19 Hughes W T & Sm th D R Infect on during in duct on of remiss on in acute lymphocytic leukemia Cancer 31 1008 1973

?0 Klastersky J Therapy of nfect ons in acut leukem a In Chemotherapy vol 1 Cl n cal aspect of infect ons Proc 9th Int Congr Chemotherapy London 1975 (ed J D W ll ams & A M Geddes) p 87 Plenum Press New York 1976

21 Kritzler R A Anuria complicat ng the treatment o leukem a Am J Med 25 532 1958

22 Luscher E F D e Akt vierung des Gerinnungssys tems im Verlauf gramnegativer Infekt onen und En dotoxäm en Fortschr Med 93 1072 1975

23 M r M A Brab n G Tang O T Leyland M J & Delamore J W Hypokalem a n acute myelo c leukemia Ann Intern Med 8? 54 197?

24 Morse E Fre re ch E J Carbovie P & Bronson W The transfus on of leukocytes from donors with chron c myelocyt c leukem a to pat ents with leuko pen a Transfus on 6 183 1966

25 Pre sler H D & Björnson S Protected environ ment n the treatment of acute leukemia Semin Oncol 3 369 1975

26 Sch mpff S C Young V M Greene W H Ver meulen G C Moody M R & W ern k P H Origin of infect on in acute nonlymphocyt c leukemia. S gnif cance of hosp tal acqu s tion of potent al patho gens Ann Intern Med 77 707 1972

27 Skipper H E Schabel F M Jr Mellett L B Montgommery J A Wilkoff L J Lloyd H H & Brockman R W Implications of biochemical cytokinetic pharmacologic and toxicologic relationships in the design of optimal therapeutic schedules Cancer Chemother Rep 54 Pt I 431 1970
28 Slyck E J Supportive care of the leukemic patient Henry Ford Hosp Med J 23 117 1975
29 Udén A M Brenning G Engstedt L Franzen S Gahrton G Gullbring B Holm G Höglund S Jameson S Killander A Killander D Lockner D Mellstedt H Palmblad J Reizenstei Skårberg K O Swedberg B Wadman B & L L-Asparaginase and prednisolone pretrea followed by rubidomycin and cytosine arabinosi induction of remission in adult patients with myeloblastic leukaemia Scand J Haematol I 1975
30 Zusman J Brown D M & Nesbit M E H phosphatemia hyperphosphaturia and hypocal in acute lymphoblastic leukemia N Engl J 289 1335 1971